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Novel Derivatizing Agents for the Determination of Methylmercury by Gas Chromatography using Electron Capture Detection

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NOVEL DERIVATIZING AGENTS FOR THE DETERMINATION OF METHYLMERCURY BY GAS CHROMATOGRAPHY USING ELECTRON CAPTURE DETECTION

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of
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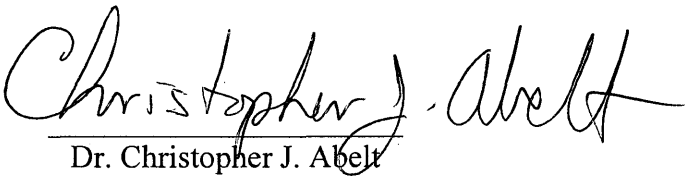
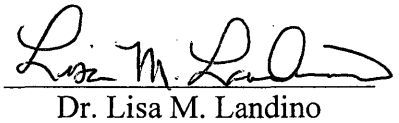
Dr. Gary Rice, Chair and Advisor



Dr. Christopher J. Abelt



Dr. Lisa M. Landino

DEDICATION

This thesis is dedicated to my parents, John and Sue Irwin, for being the best parents a girl could ask for. Thank you for your guidance, love, understanding and constant encouragement. It is also dedicated to Sean Lowe for his unfaltering support, advice and love throughout the past two and a half years.

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ABSTRACT

The accurate detection of methylmercury in fish tissue is a growing area of research. The health risks of mercury on various forms of wildlife and humans are dependant on specific forms in the environment. The most toxic forms of mercury are organic, the most prominent being methylmercury. Thus far, only total mercury concentration data is required by the FDA and EPA, partly due to methods for methylmercury detection requiring specialized instrumentation.

Currently, such methods as cold vapor atomic absorption spectroscopy (CVAAS) and gold amalgamation coupled with atomic fluorescence spectroscopy (AFS) are utilized to detect total mercury concentrations in a variety of matrices. Recent methods have included GC-AFS, GC-MIP-AES and GC-ICP-MS for the determination of methylmercury. Recent studies have demonstrated that sodium tetraphenylborate shows promise as a derivatizing agent for the detection of methylmercury using GC-MS. This study considers the use of a similar derivative bearing multiple halogens such as fluorine that could easily be detected on a GC using electron capture detectors which are common and inexpensive compared to specialized instrumentation.

In the current study, polyfluorinated reagents were synthesized and tested for their ability to produce derivatives with methylmercury. The most promising derivatizing agent was found to be sodium tetrakis[pentafluorophenyl] borate, followed by sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate. These proved quite successful for derivatizing standard solutions of methylmercury chloride with limits of detection down to ~0.8 ng/mL. Further testing must be done with actual fish samples to fully assess the application of these novel derivatizing agents.

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NOVEL DERIVATIZING AGENTS FOR THE DETERMINATION OF METHYLMERCURY BY GAS CHROMATOGRAPHY USING ELECTRON CAPTURE DETECTION

INTRODUCTION and BACKGROUND

Mercury is a highly toxic global pollutant dissipated throughout the environment, primarily by atmosphere deposition. Elemental, inorganic and organic mercury levels in the environment have significantly risen with the increase in the burning of fossil fuels, particularly coal. All forms pose various levels of threats to humans and other species, the most dangerous being from organic mercury compounds, specifically in the form of methylmercury. Methylmercury has higher toxicity, lipophilicity and volatility than other forms and accumulates easily in living organisms, especially fish exposed to water pollution.[1] Inorganic (Hg^{2+}) and methyl mercury (CH_3Hg^{+}) are the forms most commonly found in fish tissue. Current methods and protocols typically call for the determination of total mercury and thus do not accurately indicate the toxicity of the mercury relative to the specific forms, thus the development of accurate, sensitive and efficient methods for the analysis of organic mercury content in fish tissue is of critical importance.[1]

Mercury poisoning can result from acute or prolonged exposure to mercury and can affect the central nervous system, the kidneys, mucous membranes and other parts of the body depending on the type of mercury.[1] Elemental mercury can exist as a liquid or vapor at room temperature and is found in thermometers, barometers, dental amalgam, fluorescent bulbs and batteries. Environmental sources include the burning of fossil fuels (especially high-sulfur coals), chloralkali production, mercury mining and smelting, waste incinerators, crematoriums, and volcanoes. Absorption by the gastrointestinal tract is not significant, thus ingestion of small quantities of metallic mercury does not pose a

serious danger. Inhaled elemental mercury on the other hand, can ultimately enter the central nervous system and result in insomnia, forgetfulness, loss of appetite and mild tremors. Chronic exposure can result in more severe tremors and erethrism.[1] Erethrism is characterized by irritability, excitability, social withdrawl, insomnia, and anxiety.[2] Elemental mercury is not viewed as a serious threat to the public at large due to the fact that there are typically insignificant levels of mercury found in the air, as well as the fact that dental amalgams were reported by the National Institutes of Health to pose no health risks.[3]

Common salts of mercury include mercurous chloride (Hg_2Cl_2) and mercuric oxide (HgO) that have been used in a number of consumer products such as teething powders for infants and skin creams.[2] The United States has banned the use of such mercury-containing products, but they can still be found on the world market. Only a small percentage of mercury salts are typically absorbed internally, but they can be extremely caustic. In contrast to elemental mercury, the ingestion of inorganic mercury compounds can be fatal. Ingestion of significant quantities can result in gastrointestinal ulceration, perforation and hemorrhage followed by circulatory collapse. Central neuropathy and acrodynia (painful extremities) can also result.[3] Inorganic mercury accumulates primarily in the kidneys and distributes to the central nervous system only after long-term exposure because of poor lipid-solubility. Toxicity can also result from chronic dermal exposure.[1]

The most toxic forms of mercury exist in organic forms, the most prominent being methylmercury, ethylmercury and phenylmercury. Methylmercury is the most toxic as well as the most persistent of the mercury compounds bioaccumulated. Methylmercury

is formed by the methylation of inorganic mercury by sulfate reducing or methanogenic bacteria, typically in an aqueous media, or it can be formed through transmethylation reactions with organometallics.[1] Higher organisms, including humans, are mainly exposed through the consumption of fish, but organic mercury has been found in antiseptics, bactericidals, embalming agents, fungicides, germicidal agents, diaper products, paper manufacturing and wood preservatives.[1] Indeed, consumption of contaminated fish is the leading cause of methylmercury ingestion. In fish and humans, methylmercury is bound to the tissues in the body through sulfhydryl groups in proteins.[4] The fish most prone to accumulation of methylmercury tend to be those higher on the food chain, including shark, albacore tuna, mackerel, swordfish and tilefish.[3] This is because mercury in the aquatic system tends to biomagnify: a term describing the increase in concentration with each level of the food chain.[5] Once mercury-containing fish are consumed by humans, organic mercury can cross the blood-brain barrier, and in pregnant women can cross into the fetus via the placenta.[3] Children and nursing mothers are advised to limit consumption of these species in order to decrease the risk of potential neurotoxic effects.[1,3] Once inside the fetus, mercury can produce changes in the synthesis and structure of DNA and RNA in the brain, causing developmental retardation.[5]

The Food and Drug Administration mandates that fish sold commercially contain less than 1 ppm total mercury[6], while the EPA advises more conservative regulations and distinguishes between recreational (0.4 ppm) and subsistence (0.049 ppm) fishermen. The FDA rationale is based primarily on commercially sold fish consumed by the general public who seldom consume the significant amounts of fish as those who regularly fish in

local waters. If these local areas have elevated levels of contamination, local fishermen could have an increased risk over the years compared to the general public. In 1993, 29 states had mercury advisories for specific fishing areas, but within five more years, 40 states had advisories. Compared to other metals, such as arsenic with only 1 and 3 state advisories in 1993 and 1998 respectively, mercury is by far the most widespread metal contaminant, accounting for 68% of all advisories nationwide.[7]

Incidents of mercury poisoning were documented in Minamata Bay, Japan in the 1950's from ingestion of contaminated fish due to factory pollution, during a famine in Iraq in 1960 and 1970 from methylmercury- treated grain, and in the United States in 1996 from a beauty cream product from Mexico.[1] In the incidents in Minamata bay and Iraq, mothers gave birth to seemingly normal babies, but later they developed blindness, deafness, psychomotor retardation and seizures.[3] Countries like Scandinavia, for instance, began the use methylmercury-containing fungicides in the 1960s, resulting in a rise of mercury content of agricultural products, as well as in local bird populations. Areas such as the costal waters of Sweden also showed high methylmercury levels, even when methylmercury compounds were not used. This was some of the first evidence for the conversion of other forms of mercury to organomercury.[8]

Symptoms of organic mercury poisoning include paresthesia (tingling of the skin), peripheral neuropathy, cerebellar ataxia (unsteady gate), akathisia (restlessness), spasticity, memory loss, dementia, constricted vision, dysarthria (speech impairment), impaired hearing, smell and taste, tremors, and depression.[9] Due to its ability to accumulate in biological systems and its higher toxicity, organic mercury necessitates the development of accurate methods for testing.

Methods of Detection

There are two primary methods used by the EPA (Environmental Protection Agency), the EPRI (Electrical Power Research Institute) and the DOE (Department of Energy) for total mercury. These are CVAAS (cold vapor atomic absorption spectrometry) and gold amalgamation coupled with AFS (atomic fluorescence spectrometry). The CVAAS method continually samples a stream of elemental gaseous mercury and provides a real time output of concentration using the absorbance value correlated to Beer's Law. No means of atomization is needed due to the fact that mercury exists as a monoatomic vapor and has a high vapor pressure at room temperature. The sample can be reduced by stannous chloride or sodium borohydride and subsequently swept through an enclosed cell for absorbance by the hollow cathode lamp.[5] Highly specialized CVAAS systems can generate detection limits as low as 1 part per trillion (ppt) and exhibit dynamic ranges from 0.001-100 ppb Hg.[10]

A second method for total mercury involves the oxidation of all forms of mercury in the sample to Hg(II) followed by reduction to elemental mercury by a reducing agent such as stannous chloride in an aqueous solution. The mercury is removed from solution by purging with nitrogen using a bubbler chamber, and passed through a soda lime trap that removes acid vapors and moisture. The mercury in its elemental form is absorbed onto a gold amalgamation trap. The mercury is then desorbed from the trap through heating at approximately 450°C and swept into an atomic fluorescence detector by an inert carrier gas such as argon. The AFS detector monitors the fluorescence and the area of the peak is used to determine the sample's concentration through a comparison with an

established calibration curve.[11,12] Specialized atomic fluorescence detectors have detection limits of 0.2 ppt, or even 0.05 ppt in highly specialized systems.[10] These methods, however, are for determining the total concentration of mercury in samples and not for metal speciation.

The earliest methods for the detection of organomercury utilized gas chromatography coupled with an electron capture detector (GC-ECD), but low sensitivity resulted from the methylmercury being converted to methylmercury chloride for chromatographic analysis. The low sensitivity was due to the mass relationship of only one chloride relative to mercury (MW 202) as well as the partial ionic character of the bond, resulting in poor peak shapes. In methylmercury determinations the organic mercury must be solubilized from the sample without breaking the carbon-mercury bond, otherwise there is no distinction between inorganic mercury and organic mercury.[15]

Recent developments that have proven more sensitive by measuring signals generated from mercury include GC-MIP-AES (microwave induced plasma atomic emission spectrometry), GC-AFS (atomic fluorescence spectrometry) or GC-ICP-MS (inductively coupled plasma mass spectrometry).[13,14] The latter combination has recently become commercially available, though at a high cost (~$120,000 and up).[14] GC-AFS and GC-AES are both similar but AFS detectors specific for Hg are easier to use, far less expensive and more sensitive and selective.[13] However, these all still suffer from disadvantages such as laborious, time consuming procedures, contamination, loss of mercury, use of concentrated acids and lack of acceptable efficiency.

In 1993, a method was reported for the determination of inorganic and organomercury in animal tissue samples from the liver, brain and kidney. Total mercury

was first determined and then the inorganic mercury. Organic mercury levels were reported based on the difference between the two. Cold vapor atomic absorption spectrometry using sodium borohydride as a reductant was used to determine total mercury, while inorganic mercury was determined using stannous chloride as a reductant. The study reported that sodium borohydride reduced both inorganic and organic mercury, while stannous chloride showed weak reactivity towards organomercury, thus only reducing the inorganic mercury. The methods were successful when larger sample volumes and a preconcentration step were used. This method also required two procedures to determine organomercury concentrations, making it unsuitable for rapid and specific methylmercury detection.[4]

Derivatizing agents have been developed in order to distinguish between methylmercury and inorganic forms. The methylmercury reacts with the reagent and is in effect "tagged" with a second covalent organic group. The derivative can then be extracted by an organic solvent from aqueous solutions and separated by gas chromatography coupled with AFS, AES, or MS.

Derivatizing agents used thus far have included sodium borohydride, sodium tetraethylborate, sodium tetrapropylborate and sodium tetraphenylborate. Grignard reagents have also been utilized, but due to their reactivity with water, methods were found to be too burdensome. Over a decade ago, a hydride generation technique was reported using sodium borohydride but was quickly abandoned as an option due to the low thermostability of products and cumbersome sample preparation.[16] Sodium tetraethylborate was found useful for ethylating mercury in an aqueous-phase derivatizing reaction without the use of organic solvents and with a short reaction time. However,

there was difficulty in distinguishing between ethylmercury and inorganic mercury when high concentrations of inorganic mercury were present, as ethylation with sodium tetraethylborate induced the formation of additional methylmercury, skewing actual methylmercury concentrations.[16] A recent study in 2004 conducted at the University of Bayreuth in Germany, found that sodium tetra(*n*-propyl)borate also caused the formation of artifact organomercury compounds, again overestimating concentrations.[17]

In a comparative study by Cai, *et al*, it was found that phenylation was preferred over ethylation due to its lower reagent cost and ability to distinguish between organic and inorganic mercury. GC-AFS and GC-AES were found to be more sensitive than GC-MS but the latter method is still important due to its ability to confirm derivatization products through structural confirmations. Only GC-AES and GC-AFS showed good promise due to good selectivity and high sensitivity.[18]

Studies using sodium tetraphenylborate (TPB) were found to be quite promising as a derivatizing agent. The derivatization reaction proposed in a study by Cai, *et al* was as follows:

$$2NaB(C_6H_5)_4 + 2CH_3HgCl + 6\ H_2O \rightarrow 2CH_3HgC_6H_5 + 3(C_6H_5)_2 + 2B(OH)_3 + 2NaCl + 6\ HCl$$

This reaction gives a ratio of 2:3 for the production of phenyl methylmercury and the biphenyl byproduct. The derivatized compounds were extracted into hexanes and separated by GC coupled with an AFS detector to determine the methylmercury content. One advantage over sodium tetraethylborate is that sodium tetraphenylborate has a smaller pK_a, therefore it is more stable in water, and requires less care during preparation, and the reaction can be carried out over a broader pH range. The phenyl-mercury bond is

also stronger than the ethyl-mercury bond, thus providing more thermal stability. Phenylation can also be carried out over a broader pH range than ethylation. As a result, costs and precautions are lower for derivatization with $NaBPh_4$ than $NaBEt_4$.[16]

Sodium tetraphenylborate as a derivatizing agent was also examined by Costa in our lab and found to be somewhat promising using the single ion monitoring mode (SIM) on a GC - MS. The SIM mode monitors only the mass(es) specified by the operator and therefore offers a better limit of detection because more time is spent detecting those ions selected for monitoring, in this case the parent phenyl ion at a mass of 77. Utilizing this technique, an absolute detection limit of 2 pg methylmercury was attainable using a 2 μL injection, or ~2 ng/mL (ppb).[19]

Cai *et al* described derivatization procedures for fish tissue and sediment samples using the phenylborate derivatives. Glass vials were filled with 1 mL deionized water, 1.2 mL of a pH 5 buffer solution, 0.2 mL 1 % sodium tetraphenylborate ($NaBPh_4$), 1 mL hexanes and 0.1 mL of 200 pg/μL methylmercury standard solutions. Vials were shaken for 10 minutes and then centrifuged for 10 minutes. The organic phase was then separated into a 2 mL vial and dried with sodium sulfate. After the digestion of fish tissue in base solution, 0.2 mL of the digested sample solution was derivatized using the above procedure. Sediment samples were digested using a lengthier procedure and extracted using 1 mL CH_2Cl_2 and added to the previously stated derivatization procedure.[16]

This method for the derivatization of methylmercury followed by GC-AFS and exhibited detection limits of 2.3 ng MeHg/g based on 0.2 g of fish tissue and limits of 0.13 ng/g based on 5 g of sediment. These limits of detection are sufficient for

methylmercury analysis of most fish and sediment samples.[16] As a result of the recent commercial availability of instrumentation, AFS is becoming the current choice for mercury analysis, although a significant disadvantage is the dedication of the GC instrumentation to a detector specific only for mercury analysis.

Hypothesis

GC-ECD has been investigated in the past as a method for organomercury detection, but due to interference from other halogenated compounds and poor chromatographic performance using methylmercury chloride, it was dismissed as only an adequate method of detection.[16] Nevertheless, due to the electron capture detector's high sensitivity, low cost, low complexity, fast response, and common availability in environmental laboratories, it would seem to be an attractive option if the problems with specificity and sensitivity could be remedied using alternative derivatives.[20]

The hypothesis leading to this work is that if methylmercury can be reacted with a compound of similar reactive structure as sodium tetraphenylborate, but containing multiple halogenated atoms such as fluorine, the derivatives could be readily detected using ECD and the concentration of methylmercury determined at levels comparable to or better than less conventional detectors. The use of polyfluorinated species could also enhance the volatility of the derivatives and provide for reasonable elution times and determinations as well as better chromatographic characteristics than MeHgCl. Finally, ECD is a far more common detector for GC than highly specialized atomic spectroscopy systems, allowing for more widespread adaptability to methylmercury determination.

This work encompasses the synthesis of several polyfluorinated compounds and investigating their analytical merits as new derivatizing agents for determining methylmercury concentrations using GC-ECD. Numerous factors in developing synthetic strategies are presented in the experimental section, which consumed a considerable portion of the thesis work.

EXPERIMENTAL PROCEDURES and METHODS

Reagents

Methylmercuric chloride (MeHgCl) solutions were made up approximately every two days by dissolving 0.020g MeHgCl (from Sigma-Aldrich) with methanol in a 10 mL volumetric flask. The stock solution of 2000 μg/mL MeHgCl was serially diluted to obtain subsequent concentrations as needed, usually down to a concentration of 20 ng/mL MeHgCl.

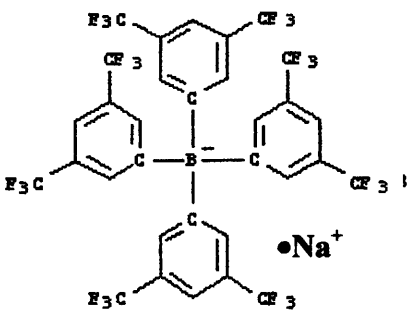
Solutions containing 1% weight/volume of synthesized derivatizing agents were prepared by dissolving either 0.050 g in deionized water in a 5 mL volumetric flask or 0.100 g in deionized water in a 10 mL flask depending on the frequency of use and amount available. Fresh solutions were sonicated for up to an hour to completely dissolve all solid and were prepared every two weeks as needed. Solutions were refrigerated when not in use. The buffer solution of pH 5 used for derivitization reactions was prepared by combining 0.20 M acetic acid and 0.20 M sodium acetate in an 18:10 ratio.

Anhydrous ethyl ether, magnesium sulfate and silicon oil (for heating) used in synthetic methods were obtained from Fisher Scientific. All other compounds and materials used in syntheses discussed below were ordered from Sigma-Aldrich Chemicals. Sodium sulfate and solvents such as methanol and hexanes used during the derivitization reactions were obtained from Fisher Scientific.

Synthetic Methods

Sodium Tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB or BARF)

Properties



The first derivatizing agent examined was sodium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate (TFPB) due to the high electrophilic character (6 fluorines on each phenyl) and the similarities in reactive properties to tetraphenyl borate (TPB). Salts of TFPB are practically insoluble in cold water and slightly soluble in hot water, as opposed to TPB which is soluble in water. TFPB readily dissolves in ethanol, methanol, ether and acetone, while less soluble in chloroform, dichloromethane and toluene. Nishida *et. al.* reported that TFPB showed no decomposition at room temperature after 1 month in 4.1 mol/dm aqueous methanolic sulfuric acid, or after seven days of heating under similar acid concentration. When exposed to air there were no signs of oxidation over a 21 hour period. In aqueous methanol at room temperature, TFPB showed a half life of 8000 days. It was shown that the trifluoromethyl groups on the phenyl ring increase resistance against both acid and air-oxidation, and increased the solubility in organic solvents along with accelerating the extraction rate.[21]

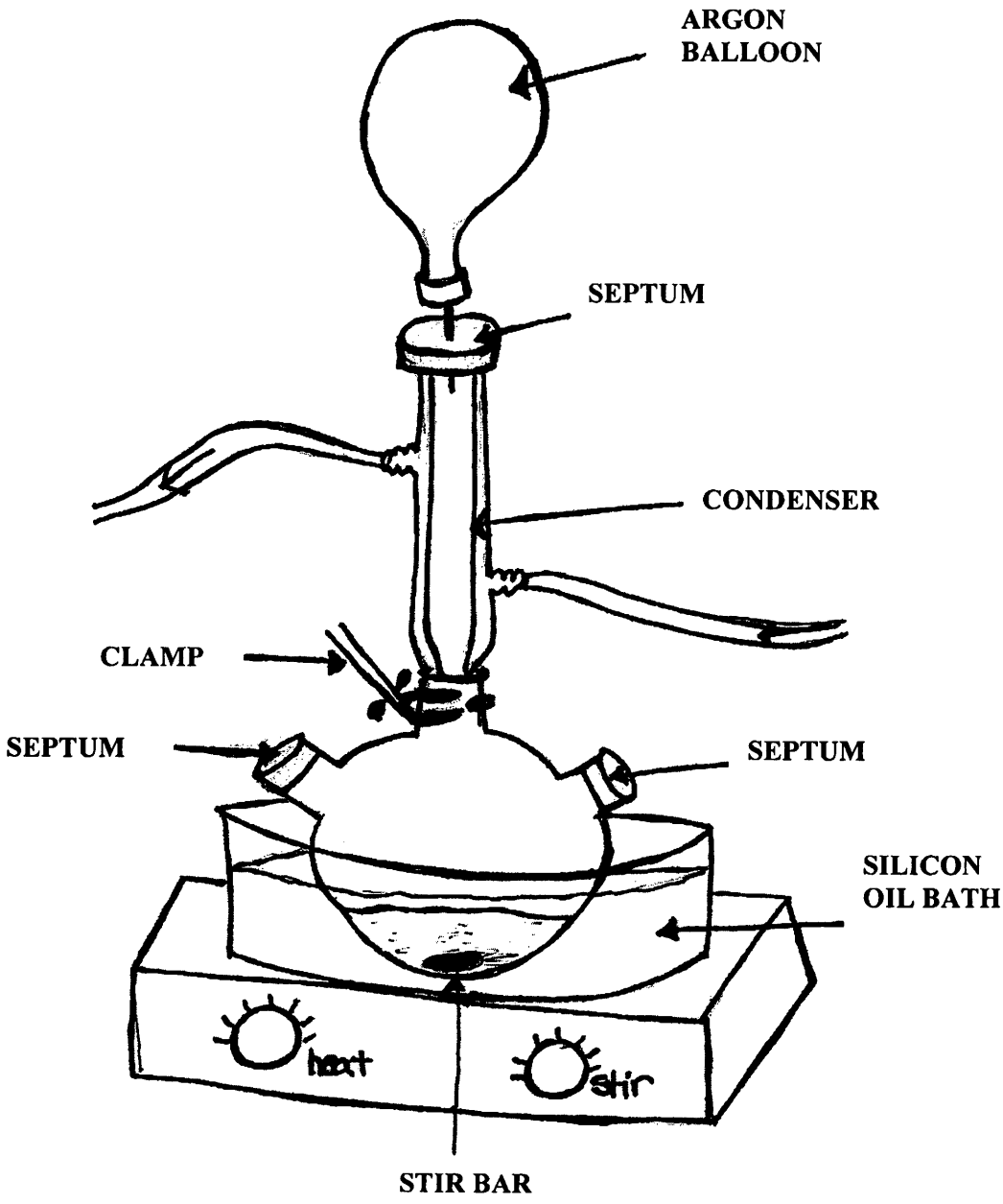
Syntheses

Due to the high cost of purchasing TFPB through Sigma-Aldrich ($800/500mg), a synthetic route was used to obtain the compound. The first scheme for the synthesis of TFPB found in the literature was reported by Fujiki *et. al.* in 1992 and involved the reaction of 3,5-bis(trifluoromethyl) iodobenzene with octylmagnesium bromide in diethyl

ether to produce a Grignard reagent. This was then reacted with BF_3• OEt_2 followed by the addition of Na_2CO_3 to form the sodium salt of tetrakis[3,5-bis(trifluoromethyl) phenyl] borate.[22] An older synthesis by Nishida *et. al.* in 1984 also reported a similar scheme in which magnesium turnings in ether were used instead of octylmagnesium bromide.[21] As the octylmagnesium bromide was already ordered, the Fujiki synthesis was attempted first.

All glassware and syringes were oven-dried at 110°C for one day in advance and subsequently cooled in a desiccator to ensure the absence of water. The setup used for the reaction is shown in Figure 1. A three-neck 50 mL flask was fitted with a condenser column with a septum for an argon balloon attachment in order to keep the reaction under an inert and anhydrous atmosphere. The other two necks were closed with septa and the round bottom was secured in an oil bath on top of a heater/stir plate. A 10 mL volume of octylmagnesium bromide (20.0 mmol, 4.349 g) was added to the round bottom using a glass syringe. A 5 mL glass syringe was then used to draw up and mix 1 mL diethyl anhydrous ether and 1.0 mL of 3,5-bis(trifluoromethyl) iodobenzene (5.64 mmol, 1.92 g). The mix was promptly added to the Grignard through the septum and then allowed to stir at room temperature for 3 hours. A mixture of 1.0 mL BF_3• OEt_2 in 7.0 mL anhydrous ether was then added dropwise. The heat was turned to the lowest setting to allow a reflux to continue overnight for a minimum of 12 hours. The mix should reflux preferably at 50°C, but on the lowest setting allowed, the oil bath reached 90°C in 1.5 hours, and then 109°C by the end of the 12 hours. At this time the solid in the flask was yellowish in color. The heat was turned off and the flask was allowed to cool while still

Figure 1. Setup of first synthesis of sodium tetrakis[3,5-bis(trifluoromethyl) phenyl] borate



stirring. The reaction was quenched by adding approximately 10 mL of aqueous Na_2CO_3 (24.0 g Na_2CO_3 diluted to 100 mL with deionized water) to the flask. Approximately 10 mL anhydrous ether was then added to attempt to put some solid back into solution. The solid was filtered using a small Buchner funnel and washed with ether. In a separatory funnel, the aqueous layer was separated from the organic layer, saturated with 10 mL of a saturated NaCl solution, and extracted using three 10 mL portions of ether. All ether portions were combined and dried with magnesium sulfate before the ether was removed using a rotary evaporator under a vacuum at 30°C. The resulting oily solution was slightly brown in color. Approximately 30 mL of hexanes were added with the expectation of a solid precipitate. The solution was then sonicated but a precipitate still did not result. The product was allowed to sit overnight in a clear beaker covered with parafilm and the solution turned a brownish grayish color, possibly from sensitivity to light. After boiling off most of the hexanes and then filtering and setting to dry, a white powder of 1.70 g resulted. A 100% yield would have given 1.25 g product (1.41 mmol), therefore it was assumed that the synthesis was not successful because of the production of extraneous by-products that would be difficult to remove.

Changes were made to the above procedure using a combination of the syntheses from Nishida *et. al.* and Fujiki *et. al.*. The iodobenzene compound was added over a period of five minutes instead of being directly added; BF_3 etherate in ether was added dropwise over 5 minutes while cooling on an ice bath in order to ensure that the reaction did not proceed too quickly. The solution was also refluxed for 19 hours instead of 12 and the resulting reaction mixture quenched with 20 mL instead of 10 mL of the Na_2CO_3 solution. After heating for 19 hours more yellow solid had formed than in the previous

synthesis. The addition of the Na_2CO_3 solution was very exothermic, thus an ice bath was added to prevent the reaction from occurring too fast. After this addition, the aqueous layer was white and contained a solid while the organic layer was yellow. Observations were the same as those of the first synthesis except a small amount of crystalline precipitate was barely visible. The product workup was the same as before, except for being left covered overnight in a freezer. This resulted in a small increase in solid, but still in an oily brown liquid that could not be successfully isolated.

During a third attempt, the Grignard reaction was allowed to sit overnight. In the morning, the solution was a light brown/tan color while before it was a grayish brown color, implying that the Grignard may not have fully formed in the previous reactions. A third synthesis reported by Bahr and Boujouk was similar to the procedure by Nishida *et. al.* but had a different product workup than the other two. In the new procedure, the resulting brown oil was shaken with benzene, the benzene was decanted off, and the oil dried in an oven at 110°C.[23] This was applied to the third synthetic workup and the resulting brown powder weighed 0.50 mg, or essentially no yield.

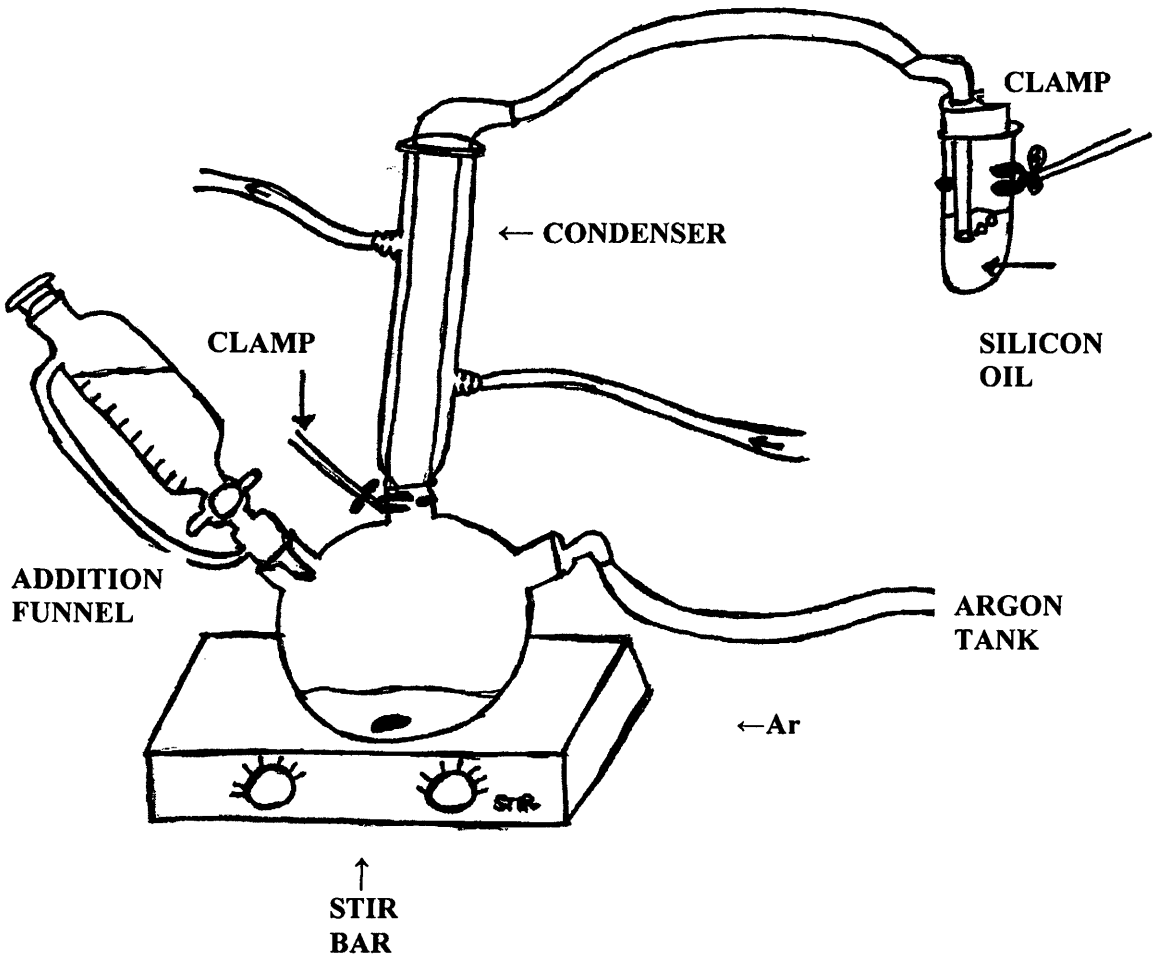
A fourth synthetic method was developed utilizing both the procedure by Bahr & Boudjouk and the first synthesis. The Grignard was produced using the iodobenzene compound but dried solid $NaBF_4$ was used instead of using $BF_3 \bullet OEt_2$. A vacuum oven was devised using an oil bath, heat plate, aspirator and small aspirator flask. The flask, containing 0.150 g (1.33 mmol) of solid $NaBF_4$, was hooked up to the aspirator in the oil bath, sealed with parafilm and a rubber stopper and subsequently heated to 110°C for at least 1 hour. After the Grignard was allowed to form overnight, the solid $NaBF_4$ was quickly dumped in to the round bottom flask and then allowed to stir for an additional 12

hours. The product after the reaction was quenched with water had markedly different characteristics from previous results. Once the water was added, a thick, milky white substance resulted that was almost impossible to extract with ether.

The fifth and final attempt used dried magnesium turnings (0.46 g, 19 mmol) and 5 mL ether added to a 100 mL three neck flask in the same set up as previously described, except with an addition funnel and a constant argon flow as shown in Figure 2. 3,5-bis(trifluoromethyl)bromobenzene (3.1 mL, 18 mmol) and 20 mL of anhydrous ethyl ether were placed in an addition funnel and added dropwise to the Mg turnings in ether over a half hour to maintain a moderate reflux. The mixture was stirred for 4 hours resulting in a dark brown solution. Solid $NaBF_4$ was dried in a vacuum oven for two hours to ensure complete dryness and then quickly dumped into the flask and the system resealed. An argon flow was maintained throughout the addition to ensure an inert atmosphere. There were no signs of an exothermic reaction at this point. After stirring overnight at room temperature, most of the ether had evaporated. More ether was added and allowed to stir in order to put some of the solid back into solution, resulting in a light brown suspension.

In a repeated synthesis, an argon balloon was affixed to the top of the column after the addition of the $NaBF_4$ and the argon flow stopped in order to reduce ether evaporation. The aqueous layer was then separated from the organic layer and extracted with three 10 mL portions of ether. The ether solutions were combined and dried with magnesium sulfate prior to removal of ether via a rotary evaporator. The resulting brown oil was shaken with benzene and the benzene decanted off. The brown oil was poured on a watch glass and partially dried by heating on a hot plate at the lowest setting (110°C)

Figure 2. Setup of final and successful synthesis of sodium tetrakis[3,5-bis (trifluoromethyl) phenyl]borate.



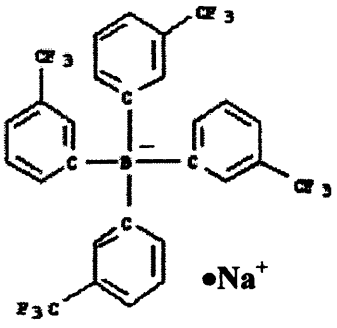
and then in an oven at 110°C to complete dryness. The resulting tan solid was further cleaned by rinsing with CH_2Cl_2 and dried again. The synthesis yielded 2.03 g of solid product, to give a 51% yield.

The powder was not totally soluble in water as stated in the literature, implying that there may have been some insoluble by-products remaining. However, all of the solid dissolved in methanol. This implied that either the literature was inaccurate or the product was something other than desired. The final synthesis yielded the only actual product that appeared suitable for preparing mercury derivatives for use on the GC-MS and GC-ECD.

The final synthesis of TFPB resulting in a 51% yield was used as a guide for the synthesis of four additional compounds. Synthetic procedures were set up in the same manner, using the setup shown in Figure 2, and including the replacement of an argon flow with an argon balloon prior to stirring for 12 hours. Starting materials were altered to produce the desired polyfluorinated reagent.

Sodium Tetrakis[3-(trifluoromethyl)phenyl]borate

Synthesis



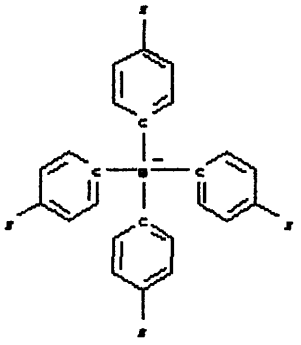
Sodium tetrakis[3-(trifluoromethyl)phenyl borate was synthesized in the same manor as the final TFPB synthesis, starting with 3-bromobenzotrifluoride (2.5 mL, 4.05 g, 18 mmol) instead of 3,5-bis(trifluoromethyl)bromobenzene. The reaction progressed as previously described until after the $NaBF_4$ was added and the mixture allowed to stir for 12 hours. The resulting solution

was a light brown color with a white suspension, only creamier looking than before. The method was the same as before, except that heating on the hot plate caused the product to start bubbling and popping. Some product was lost at this point and it took much longer to dry (approximately 1 day). The yield was 0.16 g, or approximately 6%.

Sodium Tetrakis[4-fluorophenyl]borate

Synthesis

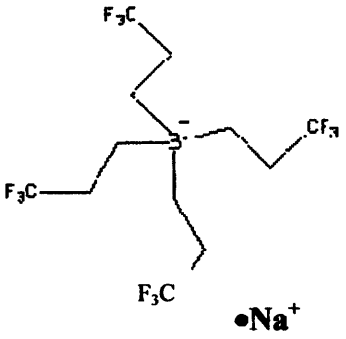
A third derivative was synthesized using the above procedure from the final synthesis of TFPB, starting with the addition of 1-bromo-4-fluorobenzene (2.11 mL, 3.798 g, 18 mmol). This is different from the previous starting materials because of the fluorine being attached directly to the phenyl. In this procedure, the solution turned brown soon after the addition of the starting material, while other syntheses took longer to develop a color. Another difference was that the resulting suspension was lighter in color after stirring for 12 hours past the $NaBF_4$ addition. The ether solution was much yellower (as opposed to brownish) than the others as well. After product workup and drying, the resulting light brown powder weighed 0.10 g, to give a 6% yield.



The solid (0.10 g) was diluted to 10 mL in methanol to produce a 1% solution. After sonicating to ensure complete mixing and allowing to settle, the resulting solution was light brown and clear but contained a fine, light cream-colored solid at the bottom of the flask, possibly due to impurities in the isolated product.

Sodium Tetrakis(trifluoropropyl) borate

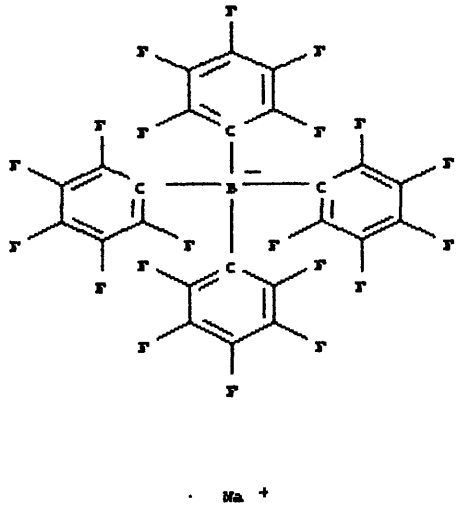
Synthesis



Sodium tetrapropyl borate has been used as a derivatizing agent in the same manner as sodium tetraphenyl borate[17], so it was assumed that sodium tetrakis(trifluoropropyl) borate could be a likely candidate. There was no synthesis found in the literature, so the previous TFPB procedure was used starting with 3-bromo-1,1,1- trifluoropropane (1.917 mL, 3.185 g, 18 mmol). The synthesis had much less color throughout the entire process than the other compounds to date. After removing the ether, the resulting solution was almost clear and was poured on a watch glass set on a hot plate to dry. The solution bubbled slightly, popped once and was removed from the heat. The popping became more violent even when removed from the heat and started to smoke. A substantial amount of product was lost during this time, and once bubbling ceased, the resulting product was white in color with black charred areas on the top. The unexpected reactivity upon drying and limited starting materials did not allow for subsequent refinement of the synthesis.

Sodium Tetrakis[pentafluorophenyl] borate

Syntheses



A final derivative was synthesized starting with bromopentafluorobenzene (2.3 mL, 4.45 g, 18 mmol). The procedure proceeded in the same manner as TFPB, but the solution turned black instead of brown. The color also appeared much faster than in the other reactions. The resulting powder was uniform and darker in color than the previous products, with a yield of 0.16 g, or 5.5%.

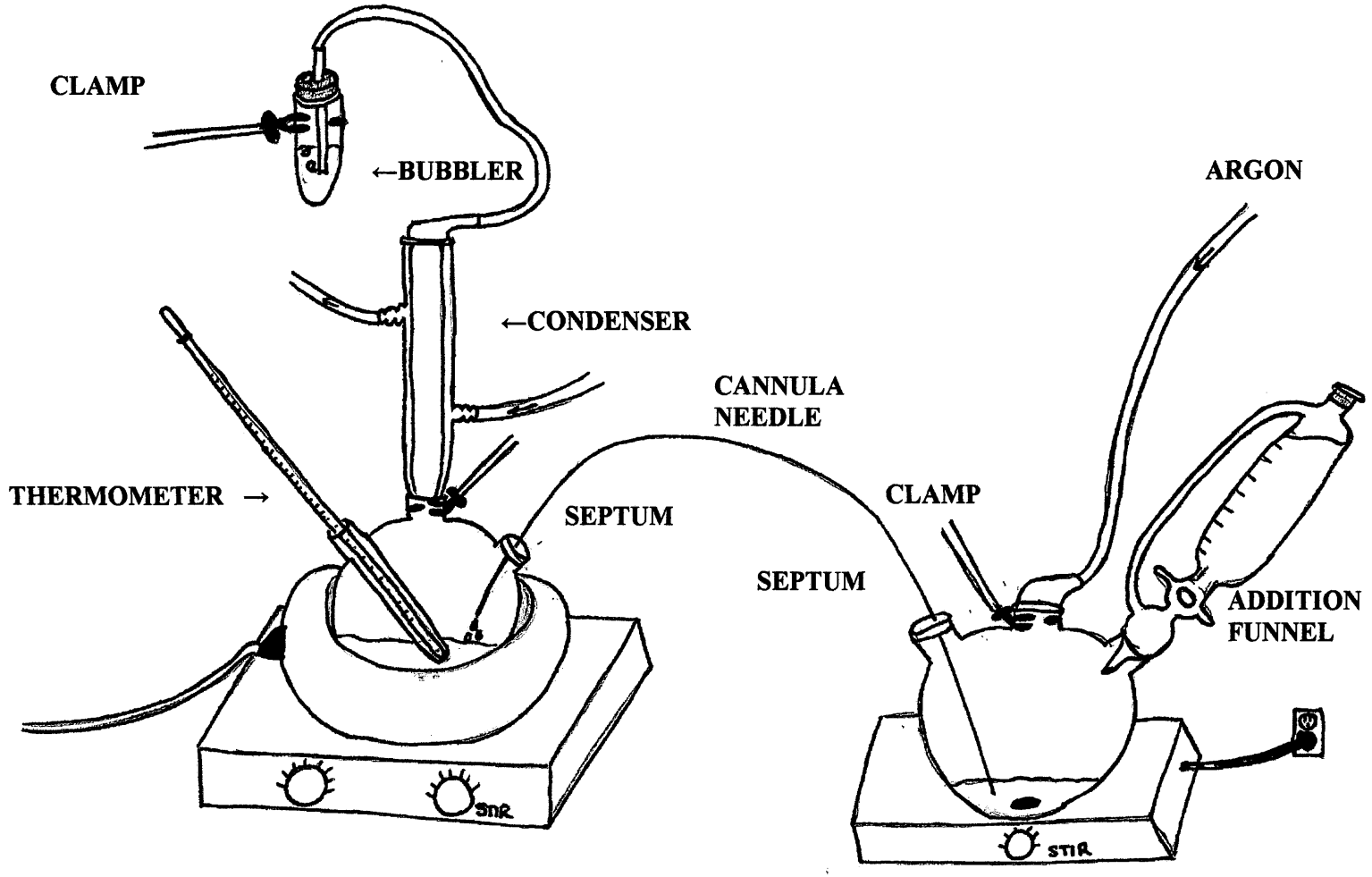
In initial testing on the GC-MS and GC-ECD, the pentafluorophenyl derivative appeared to be the most promising with respect to the detection of methylmercury concentrations. In order to improve the yield, another synthesis specifically for sodium tetrakis(pentafluorophenyl)borate was attempted. A patent from 2001 used the addition of a small amount of ethyl bromide in order to initiate the formation of the Grignard reagent due to the reactivity of the bromopentafluorobenzene and the possibility that it would be difficult to insert magnesium into the starting material.[24]

The procedure was the same as the previous synthesized compounds, but 0.3 mL (3.95 mmol) of ethyl bromide was added to the magnesium turnings in ether and allowed to stir for a half hour. The resulting solution was slightly blue and cloudy in appearance. The bromopentafluorobenzene was then added over a half hour, with the mixture turning brownish but still was a bit cloudy and light blue. After adding $NaBF_4$ and stirring for 12 hours, a grayish brown suspension resulted. The product workup was same as before and

the resulting powder was not as dark in color, but was brown and uniform in appearance. The isolated solid product weighed 0.521 g, giving a 17% yield. This yield was markedly improved from the previous synthesis, but a more efficient procedure was needed.

The patent procedure was not initially carried out due to the cost of the starting material, tris(pentafluorophenyl) borane. However, in an attempt to increase the yield, 1 g of tris(pentafluorophenyl) borane was ordered from Sigma-Aldrich. If the yield was close to the 72% noted in the patent, approximately 1 g of product should result. The setup for this modified procedure is shown in Figure 3. A 0.03 mL volume of ethyl bromide was added to 10 mL ether containing 0.0486 g magnesium and allowed to stir for 30 minutes. Bromopentafluorobenzene (0.26 mL, 0.49 g, 2 mmol) was added to the mixture over a period of 10 minutes at 25°C. This was allowed to stir for one hour to allow the magnesium to react, resulting in a slightly cloudy bluish solution similar to that observed in other syntheses. This solution was added through a cannula needle to a second 50 mL round bottom flask that contained 1 g of tris(pentafluorophenyl)borane in 10 mL of dry toluene. The mixture was heated to 65°C for 5 hours using a heating mantel to form the bromomagnesium salt. Two phases formed when this was cooled to room temperature. This solution was converted to a sodium salt through cation exchange by adding 10 mL of a solution of 20 g NaCl in 100 mL deionized water and then allowed to stir for one hour. The phases were separated and the aqueous phase extracted with five 10 mL portions of ether. The ether extracts were combined and dried with magnesium sulfate.

Figure 3. Setup of successful synthesis of sodium tetrakis[pentafluorophenyl] borate.



Most of the ether was removed on a rotovap, however an attempt to remove the rest on a vacuum line overnight did not work. The oil was re-dissolved in ether and the majority of the ether was evaporated off with an argon flow at room temperature. The oil was then put into a vacuum chamber in a small beaker and allowed to sit overnight again, and finally was put into an oven at 108°C for two days. The resulting tan, homogenous powder was scraped from the bottom and weighed. The final mass was 0.93 grams, giving a yield of 68%.

The first syntheses of the product did not fully dissolve in methanol or water, leaving a white solid that settled to the bottom. The final synthesis of sodium tetrakis[pentafluorophenyl] borate was successfully dissolved in water. A tenth of a gram of powder was diluted in 10mL deionized water and sonicated for an hour and a half to produce a clear solution with a tan tint. There was no solid remaining after sonication. The solution and powder were stored in a refrigerator when not in use.

Derivatization

A report from 2000 in *Chromatographia* used sodium tetraphenyl borate (TPB) as a derivative for the determination of organic mercury using GC- AFS.[16] The same derivatization technique was utilized here. A 1% solution of the derivatizing agent in deionized water was prepared. In the cases of the insolubility of the product in water, methanol was used. In an 8 mL screw top glass vial, 1 mL deionized water, 1 mL hexanes, 1 mL buffer, 200 μL of the derivatizing solution and 100 μL of a methylmercury chloride standard solution were mixed and shaken on a mechanical shaker for 15 minutes. The concentration of the methylmercury chloride solution was varied depending on the

calibration being used. In cases where the calibration was made by diluting the derivatized solution, the initial concentration was 2000 μg/mL MeHgCl. After mixing for 15 minutes, the vial was centrifuged for 2 minutes followed by removal of the top hexane layer with a Pastuer pipet, into a 2 mL amber vial and dried with sodium sulfate.

Instrumentation

GC-MS

Derivatized samples were initially analyzed on an Agilent GC-MS in order to determine if the derivatizing agent successfully reacted with the methyl mercury chloride, and that the specific derivatizing agent was produced during the synthesis. The instrument was set in a full ion scan mode up to a molecular weight of 450. Typical settings for the GC-MS system are given in Table 1.

GC- ECD

The results obtained from the GC-MS were used as a guide for the initial temperature settings on the GC-electron capture detector. These settings are given in Table 2. Additional parameters associated with the GC-ECD system were ultimately optimized for detection of the fluorinated derivatives. These included column flow, make-up gas and flow, injector temperature and detector temperature.

Table 1. GC-MS characteristics and parameters.

GC System

Gas chromatograph	Agilent Technologies, Model 6890N
Injection-mode / volume	Splitless / 1-2 μL
Inlet temperature / pressure	200°C
Carrier Gas	Helium
Column	15 m Length x 0.25 mm ID
Liquid phase/ thickness	100% methyl polysiloxane / 0.25μm
Solvent Delay	3.00 min
Temperature Program	80°C - 20°C/min - 280°C (5 min)

MS System

Mass spectrometer	Agilent Technologies, Model 5973N
Mass analyzer	Quadrupole
Total mass scan range	10.0 – 450.0 amu
Scans / second (typical)	4.58
Electron multiplier voltage	1500
Electron input voltage	70 eV

Table 2. GC-ECD characteristics and parameters.

GC System

Gas chromatograph	Agilent Technologies, Model 6890N
Injection-mode / volume	Splitless / 1 μL
Inlet temperature / pressure	200°C / 45.85 psi
Carrier gas	Helium
Column	Capillary 30.0 m x 320 μm
Liquid phase / thickness	HP-5 5% Phenyl Methyl Siloxane/0.25 μm
Solvent Delay	None
Temperature Program	80°C - 20°C/min - 280°C (5 min)

EC Detector

Type of Detector	μECD
Make-up gas	N_2 initially, Ar / 5% Methane where stated
Temperature	300°C
Data Rate	50 Hz (min peak width = 0.004 min)

RESULTS and DISCUSSION

I. Syntheses

Sodium Tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB or BARF)

Multiple syntheses of TFPB were performed as described in the experimental section, and ultimately the final synthesis was refined to the point of obtaining over 50% yield. The product obtained was confirmed to be for the most part TFPB by GC-MS, since it reacted with methylmercury to produce the expected derivatized product. This synthetic method was used as a starting point for all other tetraalkyl or aryl borate derivatives.

Sodium Tetrakis[4-fluorophenyl]borate

The least promising derivatizing agent synthesis was that of sodium tetrakis[4-fluorophenyl]borate. Color developed in the Grignard faster in this synthesis than previous compounds, possibly be due to higher reactivity. The resulting solid was not completely soluble in methanol or water, leaving a solid in the flask that resulted in a solution significantly less than 1% concentration of the derivatizing agent. The residual solids implied that the product has substantial impurities since the compound should have been completely soluble in methanol. The yield for this synthesis was also markedly low with 0.10 g solid, a yield of 5.8%; allowing for the preparation of only one derivatizing solution.

Sodium Tetrakis[3-(trifluoromethyl)phenyl]borate

The synthesis of sodium tetrakis[3-(trifluoromethyl)phenyl]borate was not very successful, because much of the product was lost when it was heated too fast during the drying procedure. The synthesis progressed as predicted, but the final product resulted in only 0.16 g, a yield of about 6%. Reagent costs prohibited attempting a second synthesis. The yield obtained only allowed for the preparation of one 1% solution to use for derivatization. The compound is quite similar to TFPB, less one trifluoromethyl group, thus most likely less detectable by GC-ECD.

Sodium Tetrakis(trifluoropropyl) borate

Due to the fact that there was no synthesis found in the literature for the specific synthesis of sodium tetrakis(trifluoropropyl) borate, or even a record in the literature of it existing as a compound, there was uncertainty as to whether or not the compound could be synthesized. It was postulated that the compound may show promise due to the use of sodium tetrapropyl borate as a derivatizing agent in the literature.[17] The scheme for the synthesis of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate was used as a guide for the synthesis of this compound. During the heating / drying phase some product was possibly lost and decomposed. Therefore, the derivatization results for the GC-MS and GC-ECD were not entirely reliable.

Sodium Tetrakis[pentafluorophenyl] borate

The initial procedure for the synthesis of sodium tetrakis[pentafluorophenyl] borate was modeled after the synthesis of TFPB. The first synthesis gave only 5.5% yield, while a second, modified synthesis gave a 17% yield. The product of the second synthesis was more soluble in methanol than water, but not completely soluble in either. Due to its limited productivity, a synthesis specifically designed for sodium tetrakis[pentafluorophenyl] borate was investigated as described in the experimental section. The final synthesis was carried out successfully to give a yield of 68%. The final product was completely soluble in water, so it was assumed that less byproducts were formed.

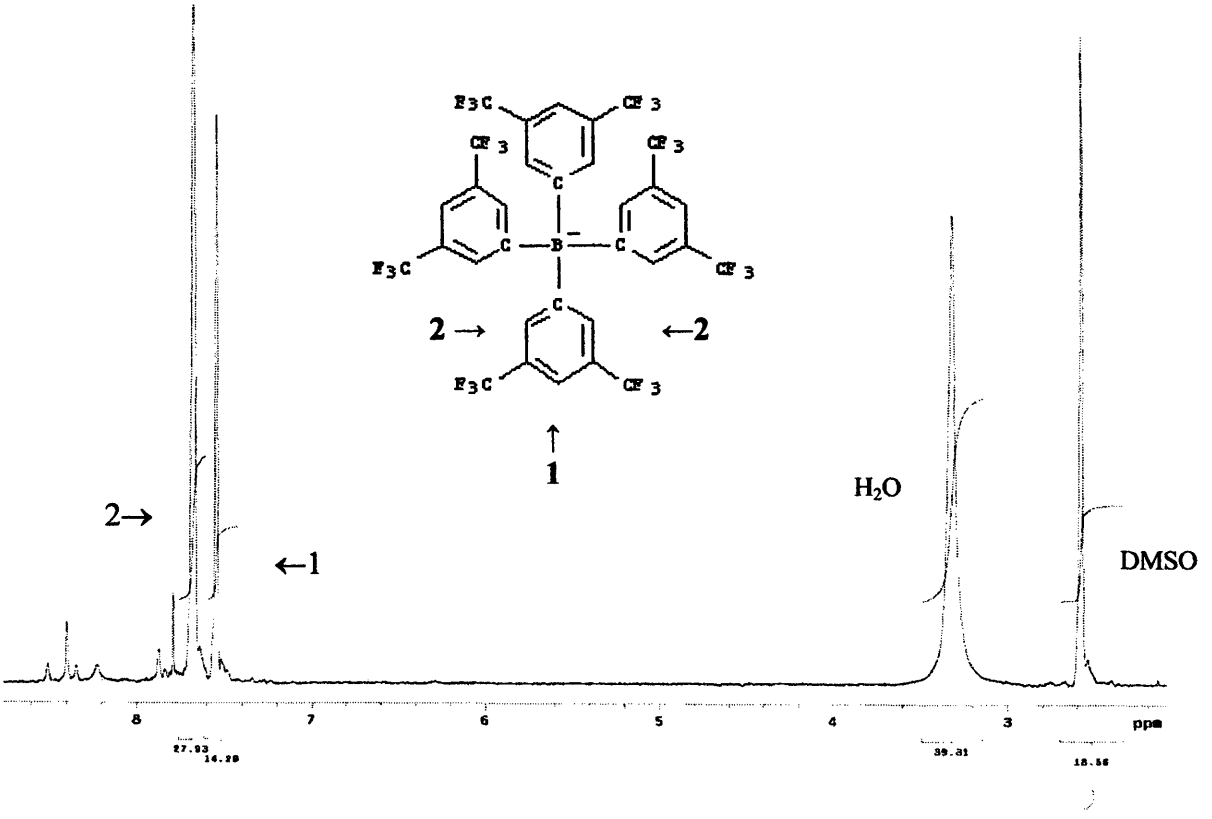
II. Product Characterization

^{1}H NMR Data

Based on data from the Bulletin of Chemistry Society of Japan (1984), there should be two singlets at 7.5 and 7.7 for TFPB.[21] The singlets should be in a ratio of 2:1 for the two different types of hydrogens on the phenyl ring. The ^{1}H NMR spectrum results were as expected for pure TFPB as shown on Figure 4. The smaller peaks shown in the chromatogram may be an indication of minor impurities in the final product.

The other compounds synthesized were not sufficiently successful or produced in large enough quantity to generate ^{1}H NMR data. The final and most promising compound, sodium tetrakis[pentafluorophenyl] borate, would not provide any ^{1}H NMR data because of the absence of hydrogens in the compound.

Figure 4. ^{1}H NMR spectrum for sodium tetrakis [3,5-bis (trifluoromethyl) phenyl] borate (TFPB).



GC-MS Data

Derivatized samples were run on the GC-MS on full scans up to 450 amu to determine if the synthesized reagents reacted with MeHgCl as hypothesized. There are seven mercury isotopes with naturally occurring abundances given in Table 3. If the reagents reacted accordingly with standard methylmercury chloride solutions, there would be a specific MS intensity pattern for any compound fragments containing mercury as illustrated in Figure 5.

Sodium Tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB)

The product from the first synthesis was derivatized using a 2000 μg/mL methylmercury chloride standard and run on the GC-MS for characterization. The chromatogram and mass spectrum are shown in Figure 6. The derivatized methylmercury compound eluted at 5.02 minutes. The methylmercury derivative produced from TFPB generated a Hg isotope pattern around 430 amu, representing the bis(trifluoromethyl) phenyl methylmercury, and a second Hg isotope pattern at 411 amu, which would represent the bis(trifluoromethyl) phenyl mercury. The most prominent peak was at 213 amu, representing the bis(trifluoromethyl) phenyl group. Figure 7 shows the chromatogram and mass spectrum of the derivatized TFPB produced from the second, more productive synthesis reported in the experimental section (no ether lost). There was a larger peak at 213 amu and the same pattern at 411 and 430 amu, but with much higher abundance. The peak at 213 amu was almost six times larger than from the first synthesis, and there was almost an order of magnitude of difference for the bis(trifluoromethyl) phenyl methylmercury molecular ion at 430 amu, implying that the product purity was

Table 3. Natural abundances of mercury isotopes.[25]

Mercury isotope (MW)	Abundance
196	0.15
198	9.97
199	16.87
200	23.10
201	13.18
202	29.86
204	6.87

Figure 5. Isotope pattern of mercury.[25]

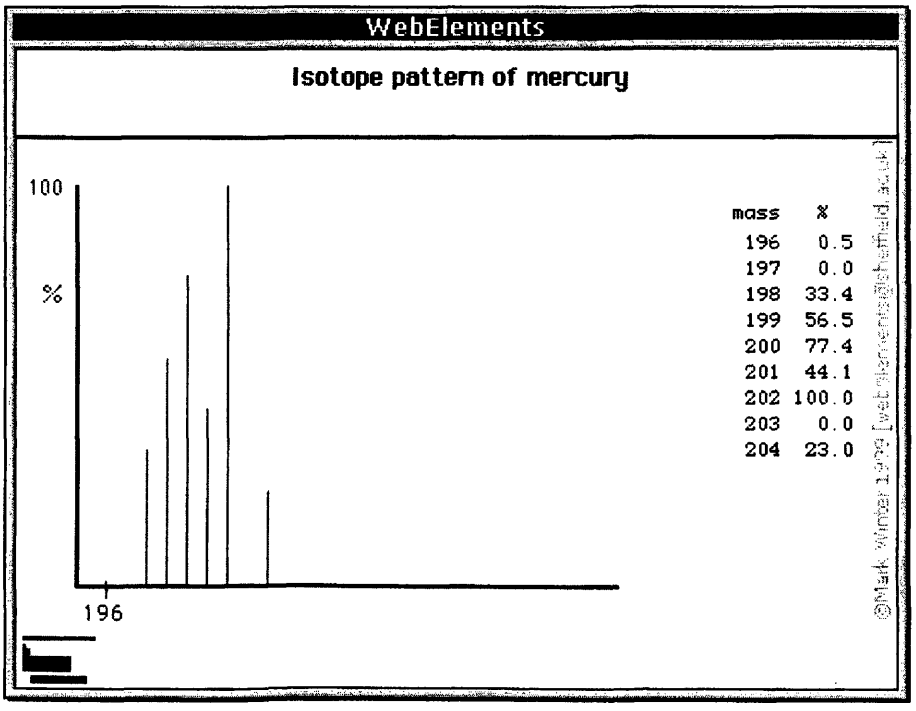


Figure 6. Full chromatogram (top) and mass spectrum (bottom) for the first synthesis of sodium tetrakis[3,5-bis(trifluoromethyl) phenyl] borate (TFPB) derivatized with methylmercury. Examination of product eluting at 5.02 minutes.

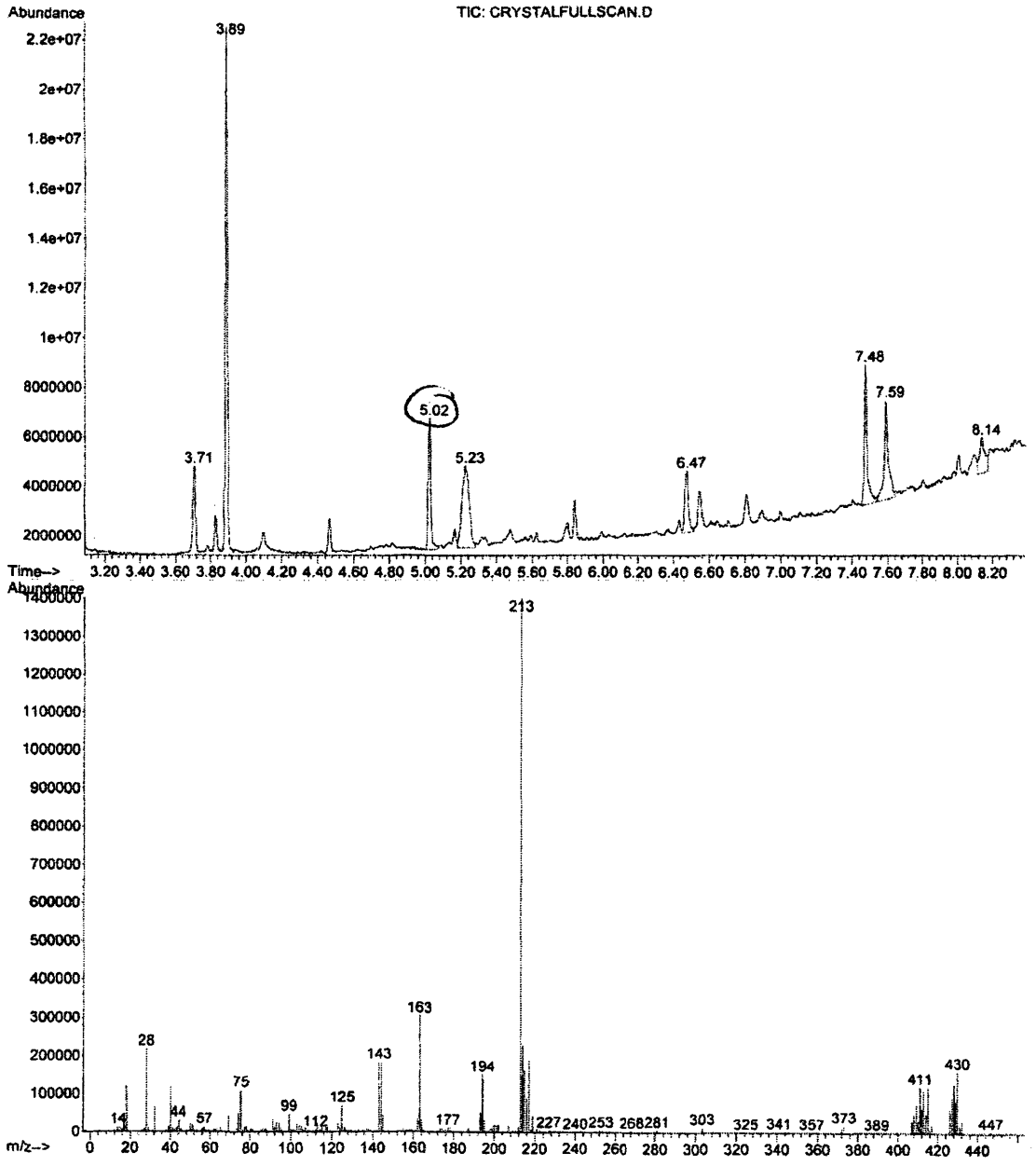
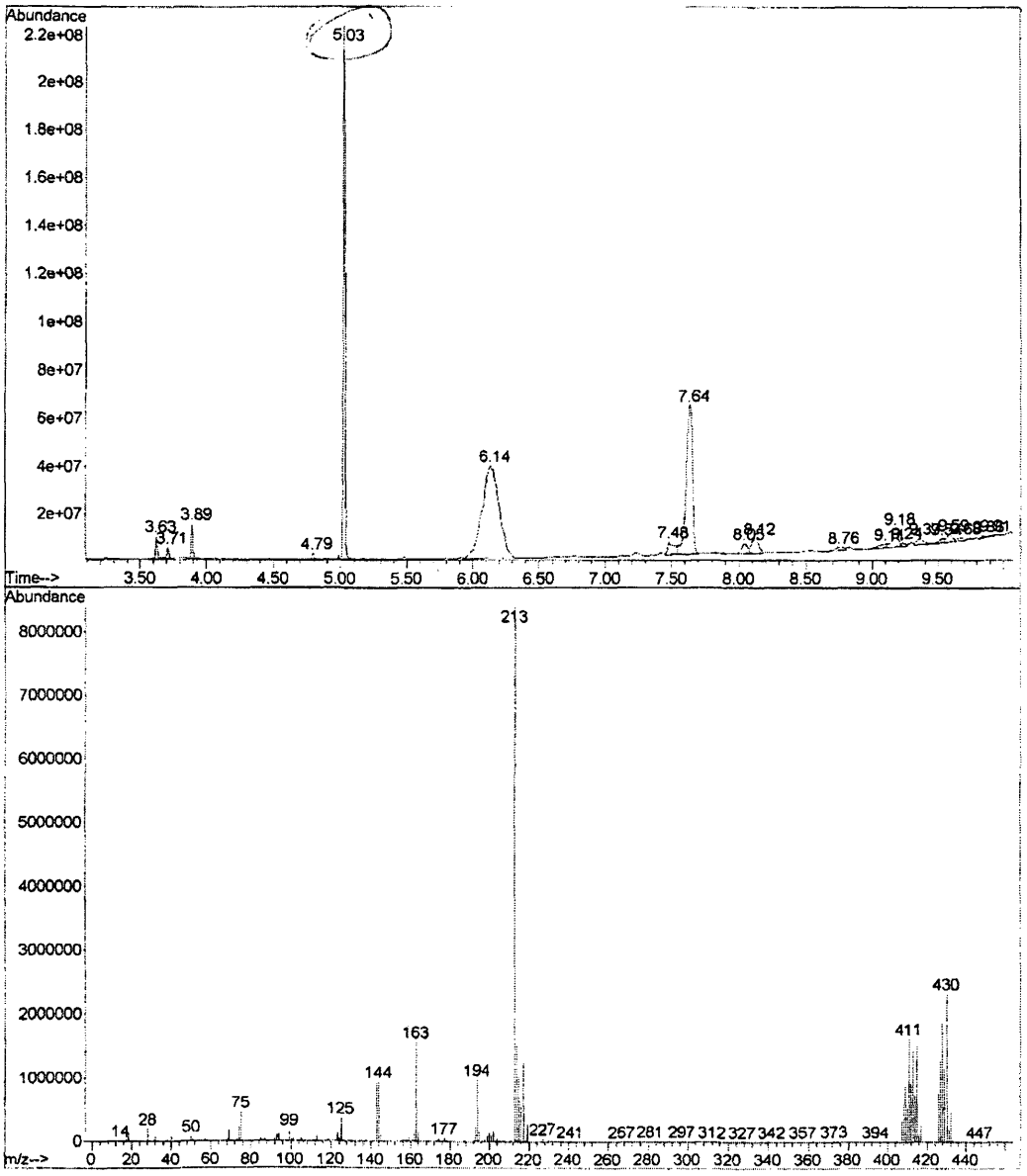


Figure 7. Full chromatogram (top) and mass spectrum (bottom) for the second synthesis of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB) derivatized with methylmercury. Examination of product eluting at 5.03 minutes.



significantly improved. There was also a significant peak that eluted at 3.89 minutes, with large mass peaks at 426 and 407 amu as shown in Figure 8. These appear to be the biphenyl byproduct that is typical from these reactions,where the 426 amu peak represents the coupling of two bis(trifluoromethyl)phenyl groups and 407 amu represents the loss of a fluorine. The peaks at 6.14 and 7.64 minutes could not be identified from the mass spectral data.

A third chromatogram and mass spectrum was obtained from the second synthesis of TFPB after being washed with CH_2Cl_2, resulting in a markedly cleaner mass spectrum as shown in Figure 9. The peak at 4.98 is the dominant peak and those at 3.5-3.8 are either byproducts or impurities and are very small in comparison. It is also evident that the patterns at 411 and 430 amu are much higher in abundance, and the impurities eluting at 6.14 and 7.64 minutes are gone.

Sodium Tetrakis[3-(trifluoromethyl)phenyl]borate

The trifluoromethyl phenyl methylmercury peaks were smaller in net abundance than those of TFPB, but were still somewhat significant as shown in Figure 10. The product eluted at 5.03 minutes, the same time as the derivatized TFPB. The Hg isotopic peak patterns were observed at 347 $(M - 15)^+$ and 362 (M^+), while the parent ion peak at 145 represented the (3-trifluoromethyl) phenyl ion. A significant peak also eluted at 4.73 minutes, as seen in Figure 11. There were significant peaks at 77 from the phenyl ring, as well as patterns at 279 and 294 amu representing phenyl methylmercury and phenylmercury respectively. Apparently there are some side reactions that result in multiple derivatized products using this reagent, precluding it from further consideration.

Figure 8. Full chromatogram (top) and mass spectrum (bottom) for the second synthesis of sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (TFPB) derivatized with methylmercury. Examination of product eluting at 3.89 minutes.

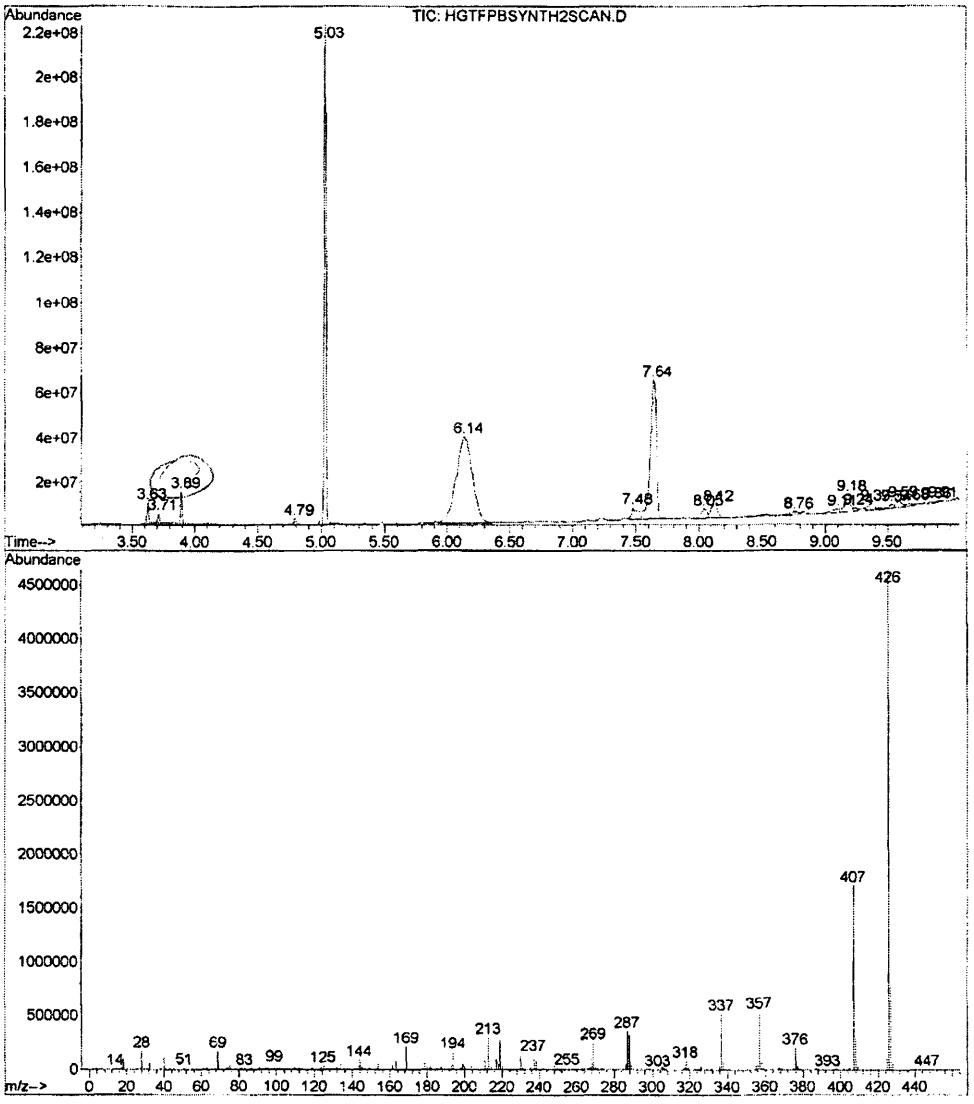


Figure 9. Full chromatogram (top) and mass spectrum (bottom) of TFPB after washing with CH_2Cl_2 and derivatized with methylmercury. Examination of product eluting at 4.98 minutes.

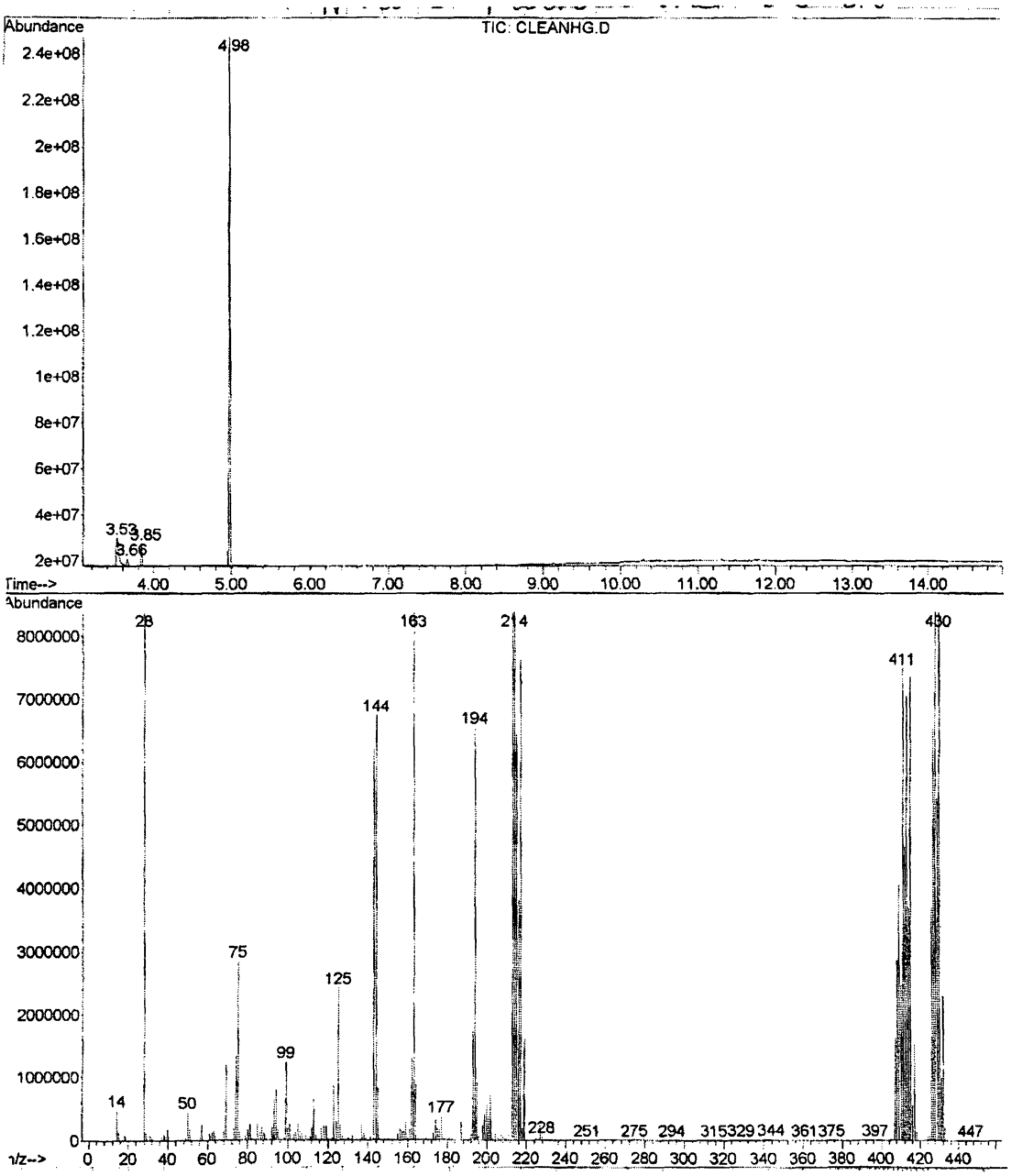


Figure 10. Full chromatogram (top) and mass spectrum (bottom) of sodium tetrakis[3-(trifluoromethyl)phenyl]borate derivatized with methylmercury. Examination of product eluting at 5.03.

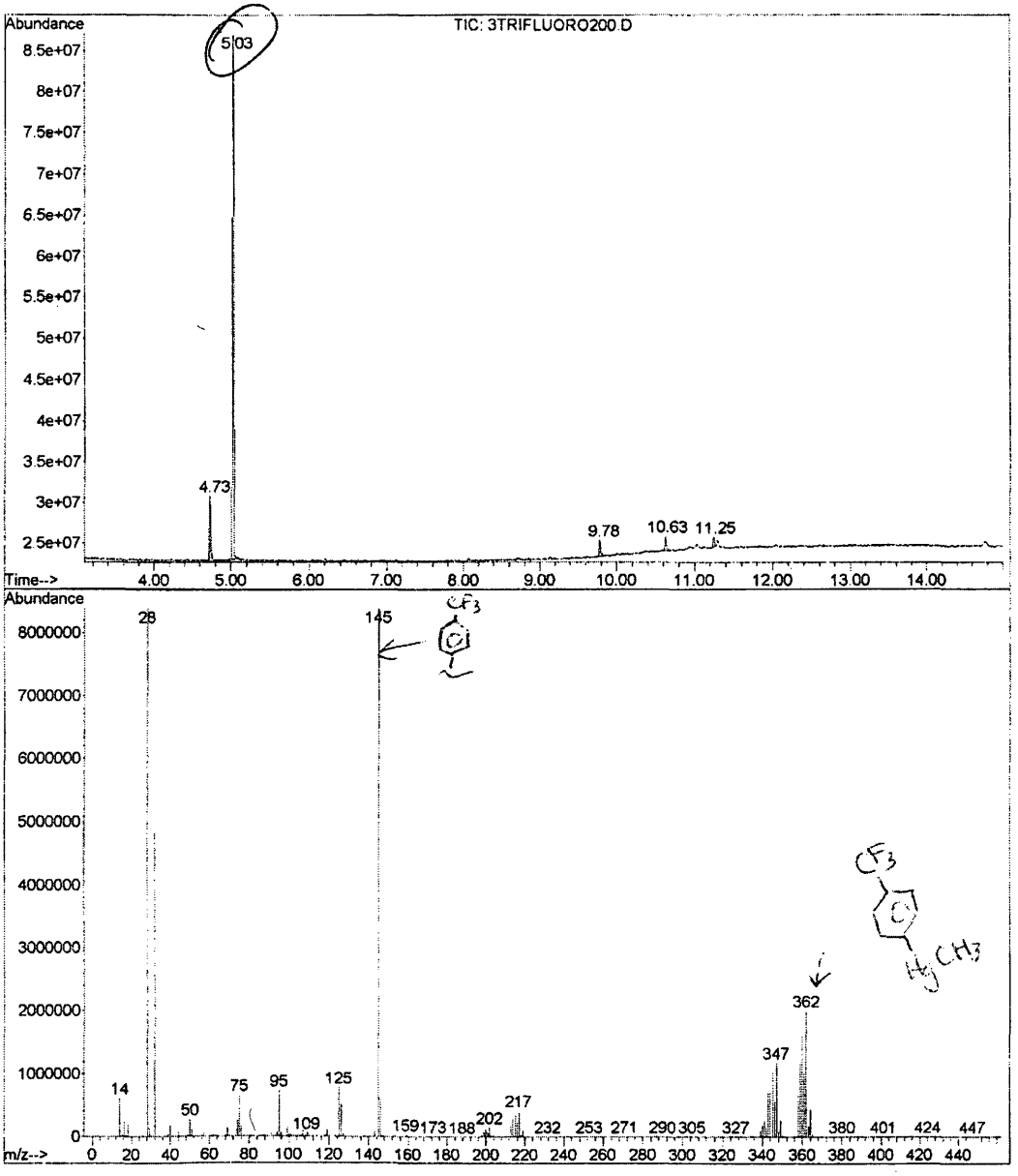
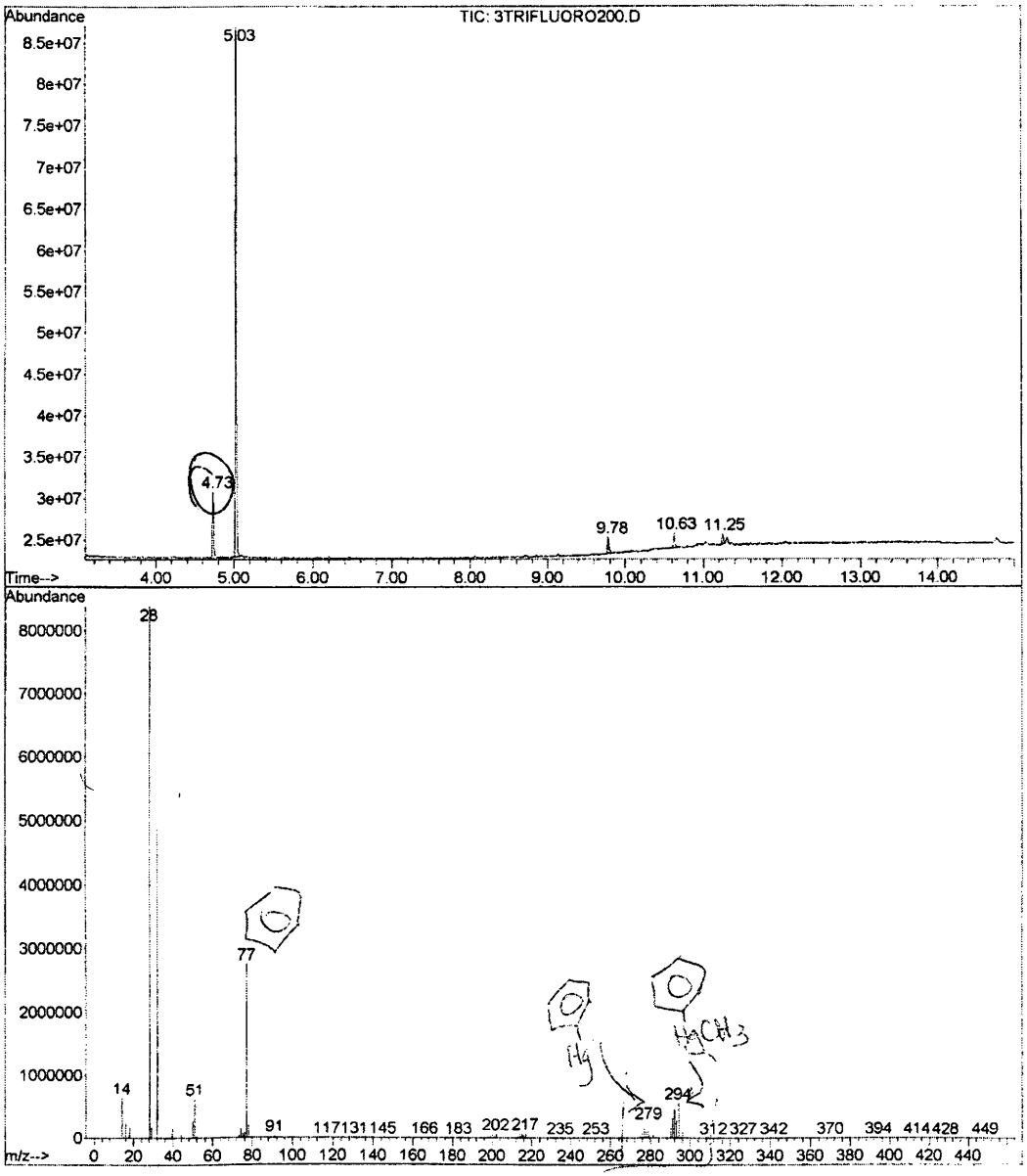


Figure 11. Full chromatogram (top) and mass spectrum (bottom) of sodium tetrakis[3-(trifluoromethyl)phenyl]borate derivatized with methylmercury. Examination of product eluting at 4.73.



Sodium Tetrakis[4-fluorophenyl]borate

The chromatogram from the GC-MS was very complex as shown in Figure 12, possibly from a higher occurrence of byproducts from the synthesis. The desired product eluted at 4.85 minutes, but there were also a number of peaks between 9 and 11 minutes, the largest being at 10.23 min. No peaks between these times could be identified from the mass spectra and were likely the result of contaminations on the column. At 4.85 minutes, the peak at 95 amu represents the fluorophenyl peak, and the patterns at 295 and 312 represent the derivatized methylmercury and mercury ions respectively.

Sodium tetrakis[trifluoropropyl] borate

The GC-MS of this compound eluted a small peak at 3.13 minutes as shown in Figure 13. A peak at 97 amu indicated the trifluoropropyl group, while a peak at 77 indicated a phenyl ring. The product shouldn't contain any phenyl ions, therefore this was most likely from contamination. There was a definite mercury pattern at 299, while the methyl mercury pattern at 312 was much less significant. There was a peak at 4.97 minutes containing the classic TPB mercury derivatized peaks- 77, 279 and 294. This was possibly due to contamination from previous injections of TPB on the column. The relatively poor sensitivity for the derivative indicated that the product was most likely very contaminated with byproducts.

Figure 12. Full chromatogram (top) and mass spectrum (bottom) of sodium tetrakis[4-fluorophenyl]borate derivatized with methylmercury. Examination of product eluting at 4.85 minutes.

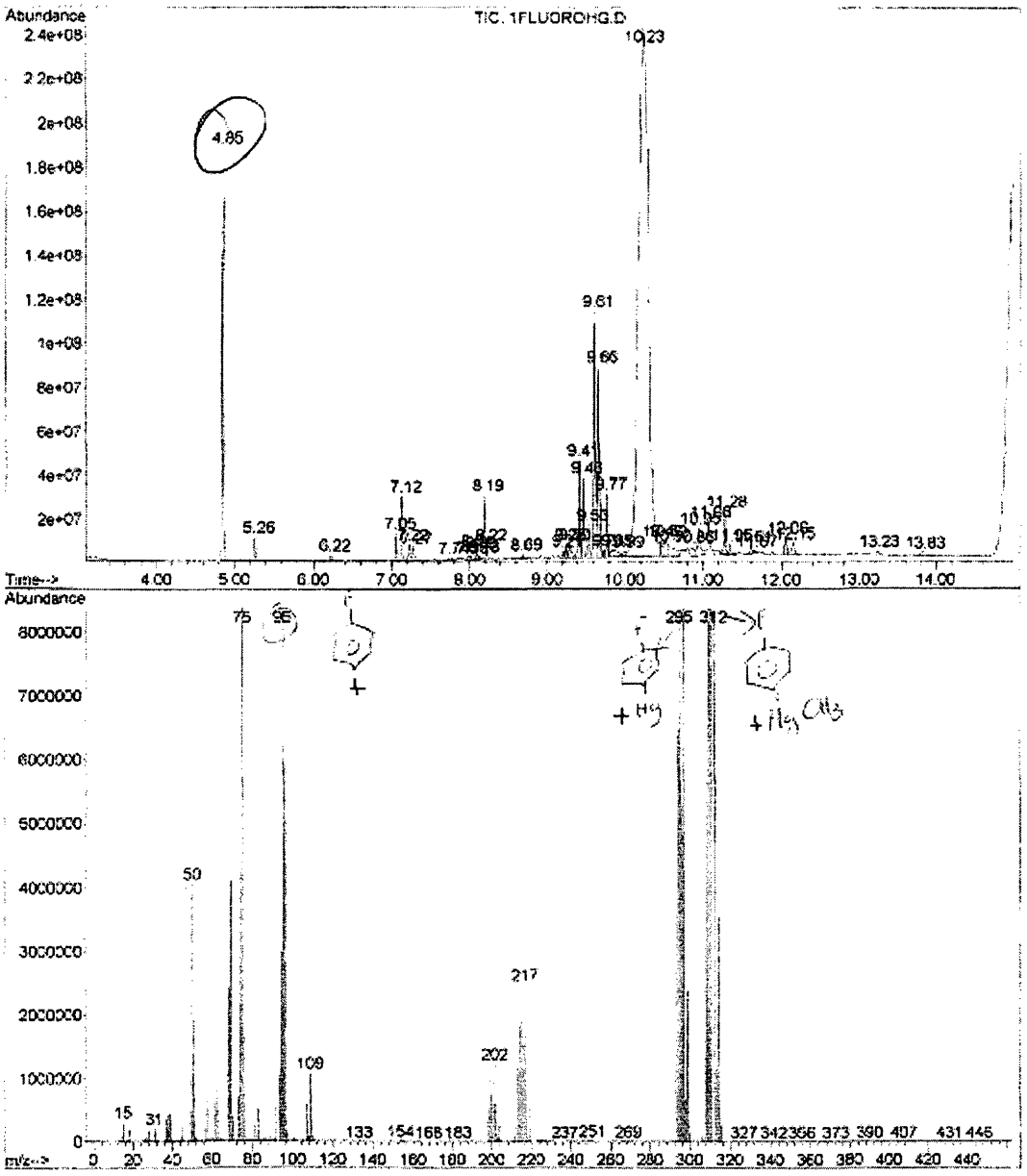
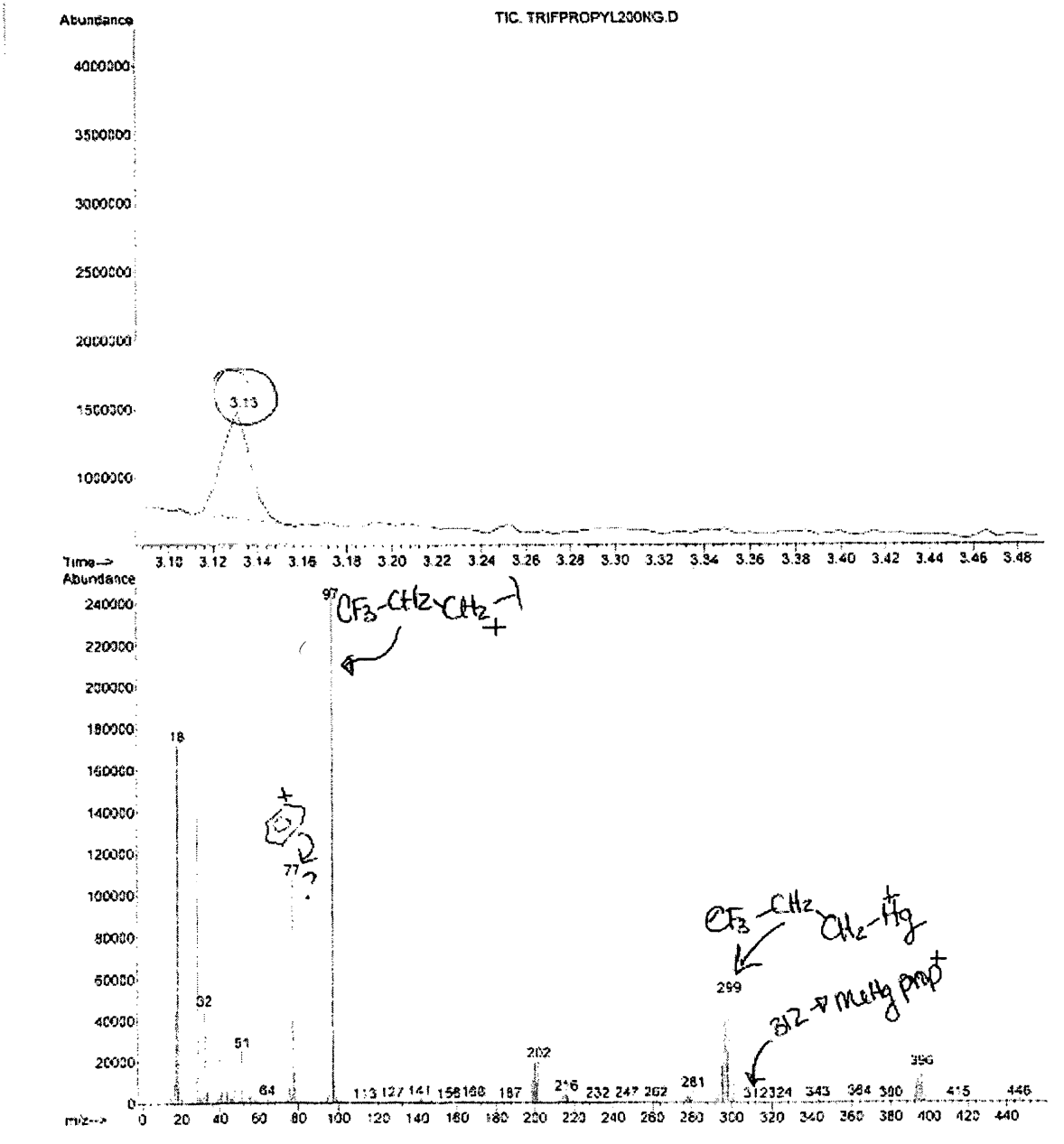


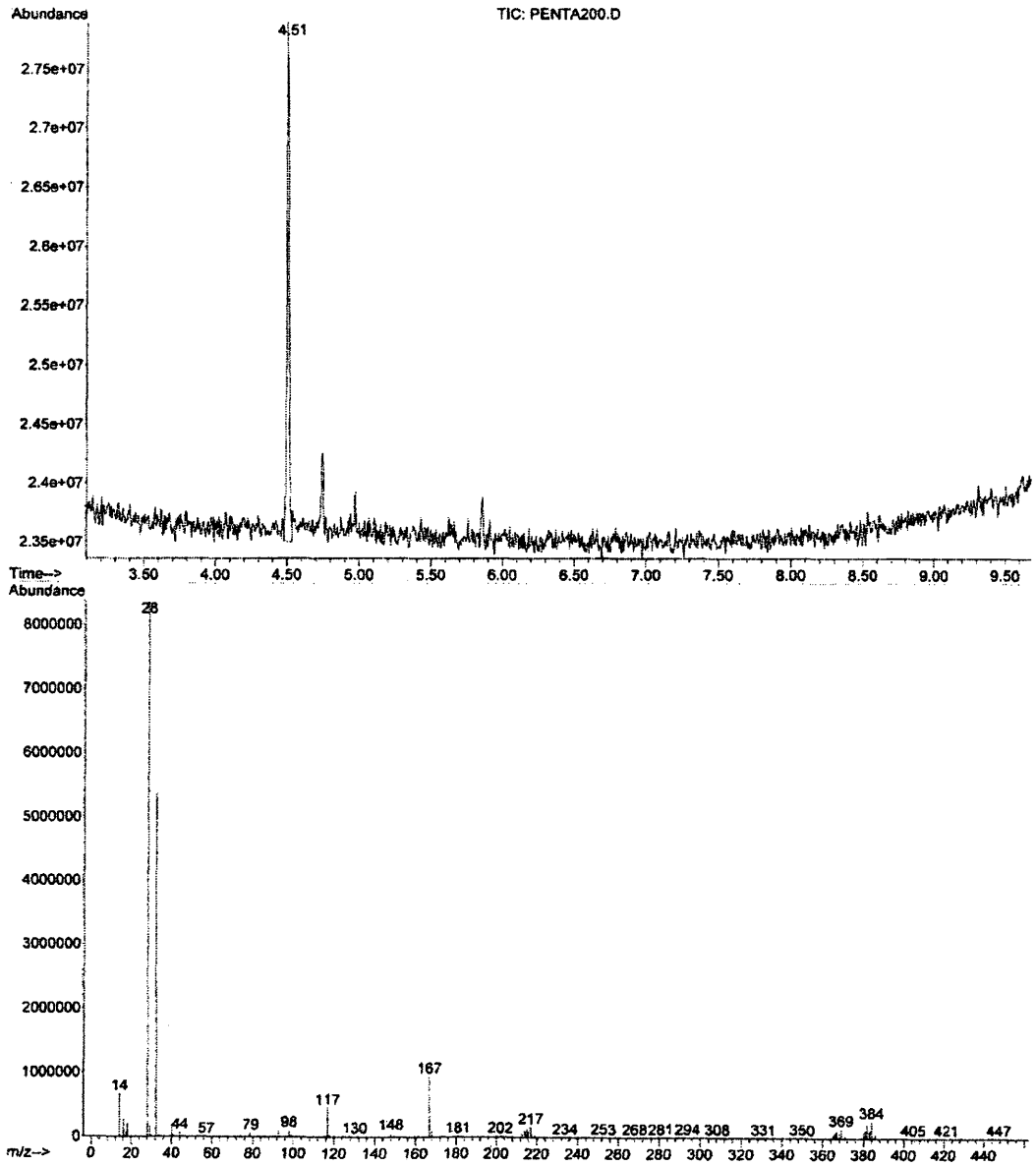
Figure 13. Full chromatogram (top) and mass spectrum (bottom) of sodium tetrakis[trifluoropropyl] borate derivatized with methylmercury. Absolute injection of 4 ng trifluoropropyl methylmercury. Examination of product eluting at 3.13 minutes.



Sodium Tetrakis[pentafluorophenyl] borate

The first synthesis produced was found to contain the desired products on the GC-MS but peak heights were much smaller than in TFPB and 3-trifluoromethyl phenyl analyses as shown in Figure 14. The product eluted at 4.51 minutes, with the peak at 167 representing the pentafluorophenyl group and the patterns at 369 and 384 representing the mercury and methylmercury pentafluorophenyl compounds respectively. A peak eluting at 3.40 minutes was determined to be the decafluorobiphenyl byproduct (334 amu). The final, more productive synthesis gave the same results, but with higher abundances of methylmercury and mercury peaks in the mass spectrum and less impurity peaks in the chromatogram.

Figure 14. Full chromatogram (top) and mass spectrum (bottom) of the first synthesis of sodium tetrakis[pentafluorophenyl] borate derivatized with methylmercury. Injection of 40 ng pentafluorophenyl methylmercury. Examination of product eluting at 4.51 minutes.



III. Analytical Merits

Evaluation of Analytical Merits Using GC-ECD

A problem associated with chromatograms obtained from the derivatives on the GC-ECD is that a large number of peaks were observed, making it hard to determine the exact peak that represented the derivatized MeHg. The presence of these unidentifiable peaks on the chromatograms led to the examination of possible sources. The methanol and hexanes used in the reaction derivatization were tested on the GC-ECD to see if any of the peaks in the chromatogram resulted from impurities in the solvents. Neither was found to contribute any significant peaks to the chromatogram.

Subsequently, a pure, highly electronegative compound, trifluoroacetophenone, was diluted in hexanes and injected on the GC-ECD to observe the number of peaks produced. It was important to determine if the high number of chromatographic peaks was from possible impurities in the derivatizing agent, as it was hypothesized that a pure highly electronegative compound would produce a clean chromatogram with only one peak. Trifluoroacetophenone was diluted in hexanes to make two samples, one 5 μg/mL and the other 500 ng/mL resulting in 5 ng and 500 pg absolute for a 1 μL injection. No peak was observed in the first 10 minutes for the first sample, but small peaks were observed from 10.62 minutes and after. A clear peak at 1.16 minutes was observed for the higher concentration, and also included smaller peaks after the 10 minute mark, possibly from some sort of contamination, or possibly from column contaminations.

Products

Sodium Tetrakis[3,5-bis(trifluoromethyl)phenyl]borate

In order to rectify this problem with identification of the retention time of the derivative, a sample containing no MeHg and just the derivatizing agent was injected, followed by a 1 μL sample containing 20 ng MeHg and another containing 30 ng MeHg that were derivatized with TFPB. The comparison is shown in Figure 15. Differences in the areas of the chromatographic peaks at 3.53 minutes could be assumed to result from the lack of derivatized MeHg in the first sample. This test resulted in the identification of two possible methyl mercury peaks at ~3.53 and ~7.16 minutes for TFPB. Figure 16 exhibits the calibration curve resulting from concentrations of MeHg ranging from 2 pg to 20 ng absolute for a 1 μL injection. It was still evident that the peak at 3.53 represented the derivatized TFPB methylmercury peak, but as of yet there was no clear explanation, other than impurities and byproducts, for the large number of peaks in the chromatogram.

The integrated peaks at lower concentrations were not very reproducible, but this could be from a lack of sensitivity at this point in time (prior to optimization of parameters). Nevertheless, it is evident that at higher concentrations, the difference between areas at 0.2 ng MeHg/μL, 2 ng MeHg/μL and 20 ng MeHg/μL is approximately a factor of 10 (as expected) for the peak at 3.53 minutes and not for the peak at 7.16 minutes.

The tetraphenylborate (TPB) derivative was observed to have a slightly faster eluting time on the GC-MS than TFPB for the same type of capillary column, so it can be assumed that a similar eluting time ratio would be observed on the ECD.

Figure 15. Determination of derivatized methylmercury peak (for TFPB) in GC-ECD.

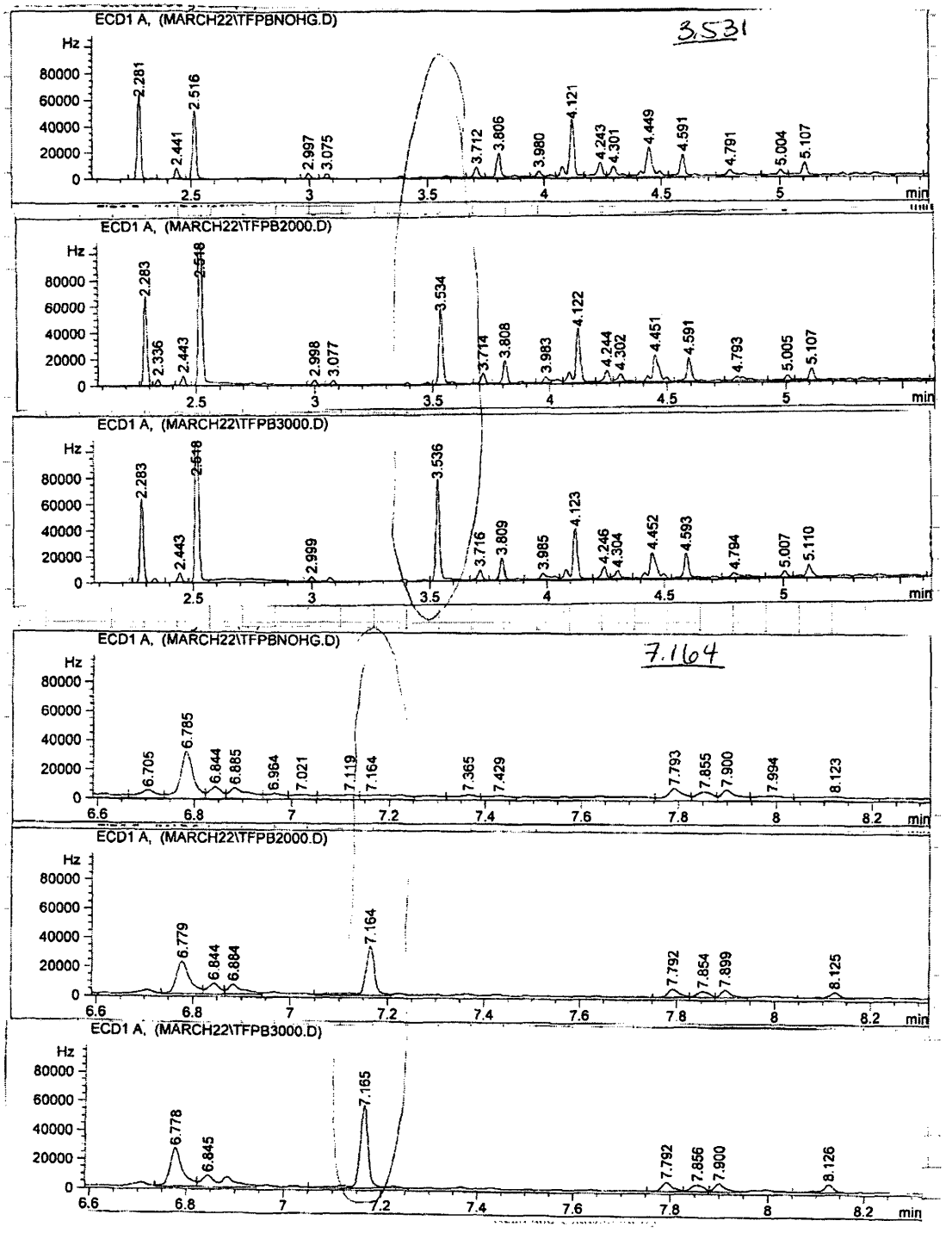
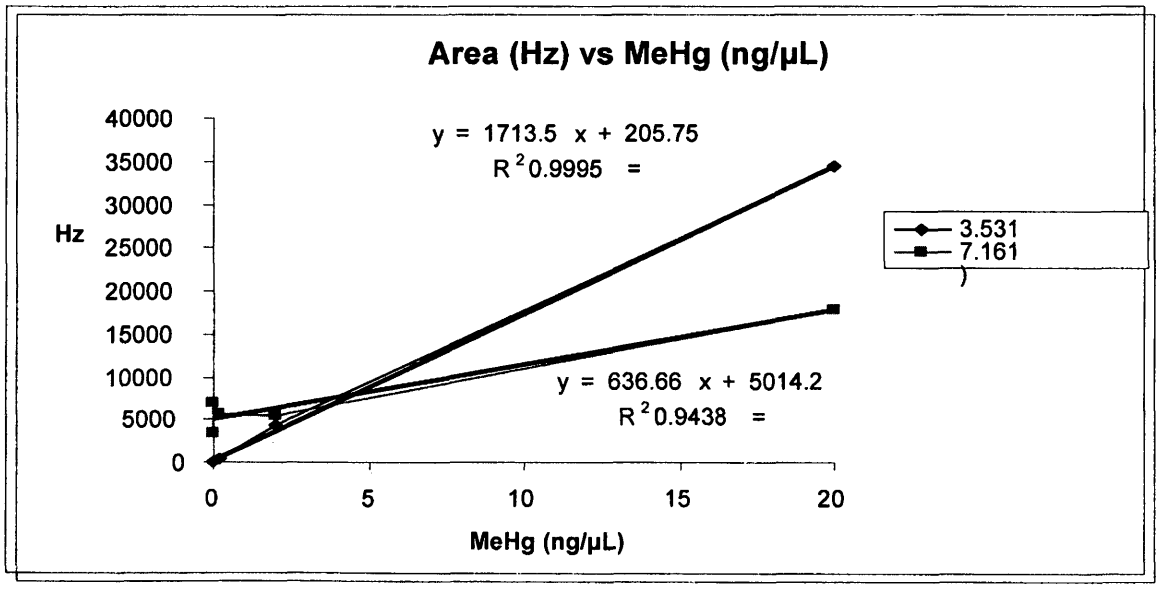


Figure 16. Calibration #1 on ECD using sodium tetrakis[3,5-bis(trifluoromethyl) phenyl]borate as a derivatizing agent.

MeHg (ng/μL)	Area (Hz) of peak at 3.531 min	Area (Hz) of peak at 7.161 min
0.002	0.025	3346.3
0.02	0.047	6934.1
0.2	465.5	5649.8
2	4222.7	5466
20	34417.5	17822.8



TPB was derivatized at a very high concentration (200 ng/μL) of MeHgCl and injected on the GC-ECD. The peak eluted at approximately 3.25 minutes, slightly faster than TFPB. This retention was absent in a run where the TPB was not derivatized with MeHgCl. From these tests it was determined that the bis(trifluoromethyl) phenyl methylmercury peak eluted at 3.53 minutes.

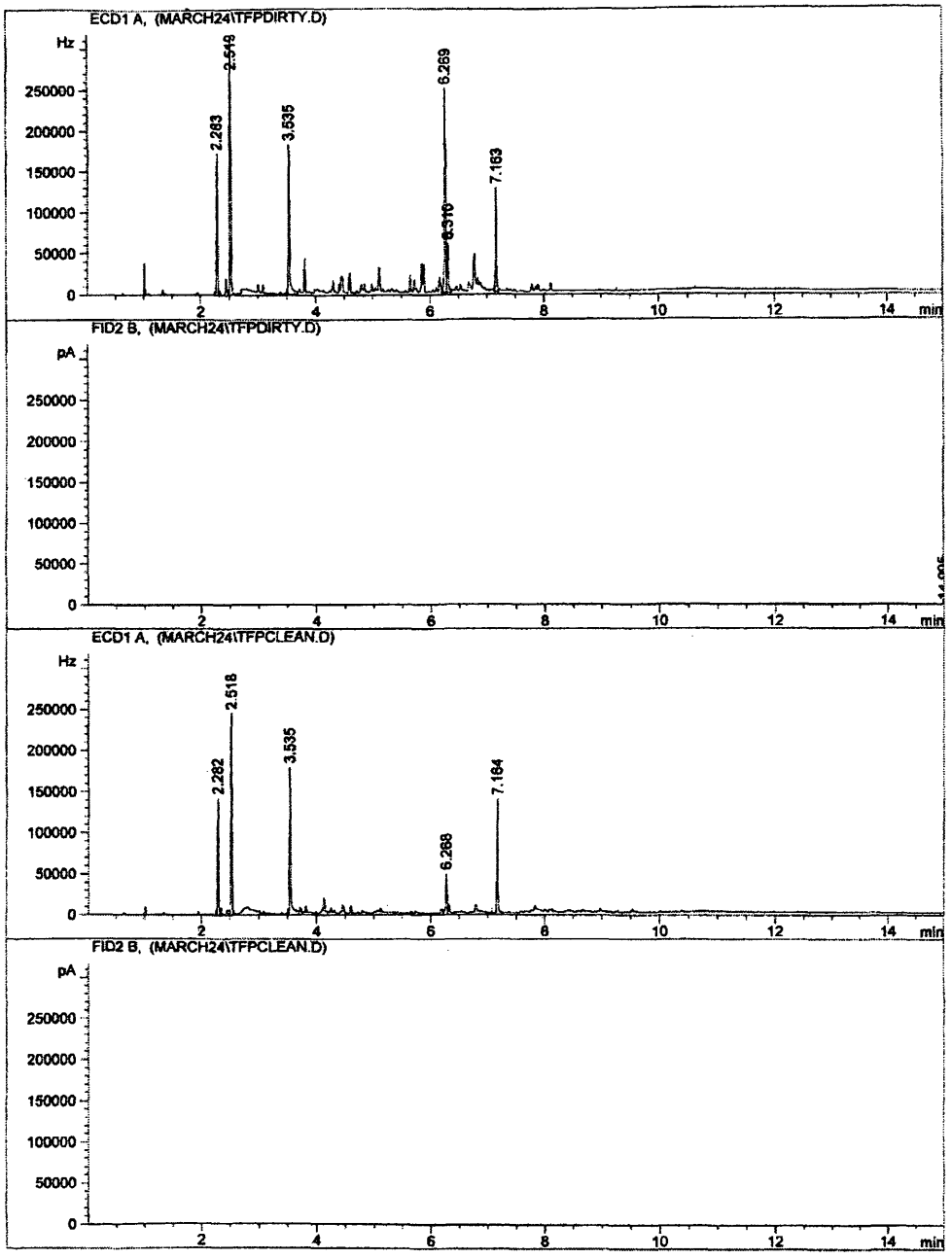
Once the methylmercury peak was determined to be at 3.53 minutes, the retention times for the rest of the derivatizing agents could be correlated from GC-MS runs for identification on the GC-ECD.

The comparison between the derivatized TFPB prior to and after washing with 5 mL of CH_2Cl_2 is shown in Figure 17. The second chromatogram was markedly cleaner, although there were still three significant peaks at 2.28, 2.57 and 7.16 minutes, implying that either there were still impurities remaining in the TFPB or other unknown derivatives were formed in the process. These peaks were not observed in full scan GC-MS runs.

Sodium Tetrakis[3-(trifluoromethyl)phenyl]borate

The derivatized reagent with and without methylmercury chloride was run on the GC-ECD. A peak was found at 3.53 minutes, very similar to that found for derivatized TFPB. The peak area in Hz however was much smaller for equal concentrations of MeHg when compared to TFPB either the result of poor sensitivity or purity of the derivatizing reagent in the 1% solution. Therefore, sodium tetrakis[3-(trifluoromethyl) phenyl]borate was ruled out as a potential derivatizing agent.

Figure 17. GC-ECD chromatogram comparison of derivatized TFPB prior to and after cleaning with CH_2Cl_2.



Sodium Tetrakis[4-fluorophenyl]borate

After running derivatizations with and without mercury, two potential methylmercury peaks were observed at 1.67 and 3.32 minutes. Peak areas were 578013 and 31249 Hz respectively, a ratio of approximately 18 to 1. The chromatogram did not contain nearly as many peaks as other derivatives, but peaks were not significant enough in comparison to other compounds. Also, due to the small yield of the synthesis, further determinations using sodium tetrakis[4-fluorophenyl]borate could not be pursued.

Sodium Tetrakis[pentafluorophenyl] borate

Reactions were once again run with and without methylmercury chloride prior to chromatographic examination. A definitive peak was observed at 3.02 minutes, which makes sense due to the fact that the compound contains a higher percentage of fluorines per mass unit than TFPB does and should be more volatile. The first abbreviated calibration using this agent was found to be quite promising. Absolute masses of 20 pg, 2 ng and 200 ng MeHg were injected onto the GC using 1 μL volumes. The calibration is shown in Figure 18. The area ratios were reasonably close to the expected value of 100.

Following calibrations proved to be less satisfactory. A calibration with additional concentrations was made the following day as shown in Figure 19. The peak at 200 fg/μL was nonexistent, while the one at 2 pg/μL exhibited some contamination. It is also evident that there was less linearity at lower concentrations as a better R^2 value was obtained for the graph of the entire calibration rather than the smaller range.

A third calibration was also unacceptable. It is possible that the partial insolubility of the product in methanol provided for discrepancies in calibrations.

Figure 18. ECD abbreviated calibration #1 for sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent.

Me Hg (ng/µL)	Area (Hz)	Height (Hz)	A ratio	H ratio
0.02	36.5	37.4	120.7	117.8
2	4406.5	4406.5	85.3	81.0
200	375686	356845		

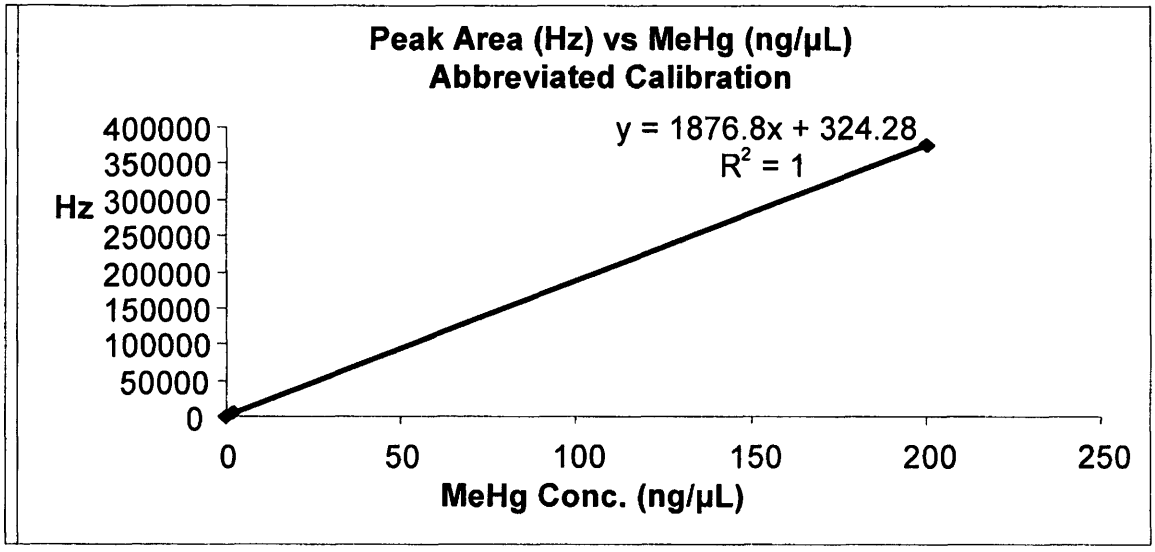
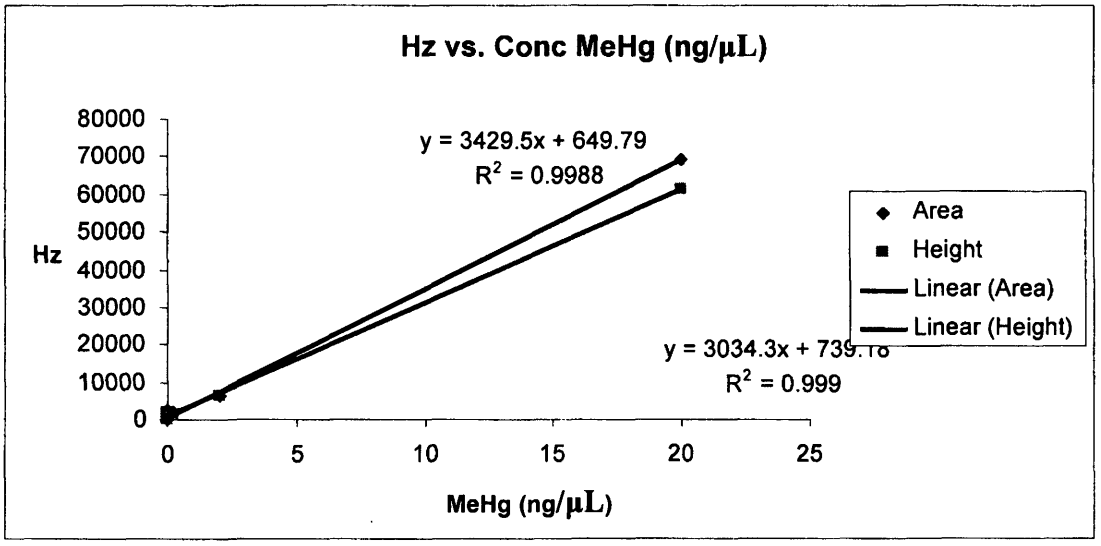
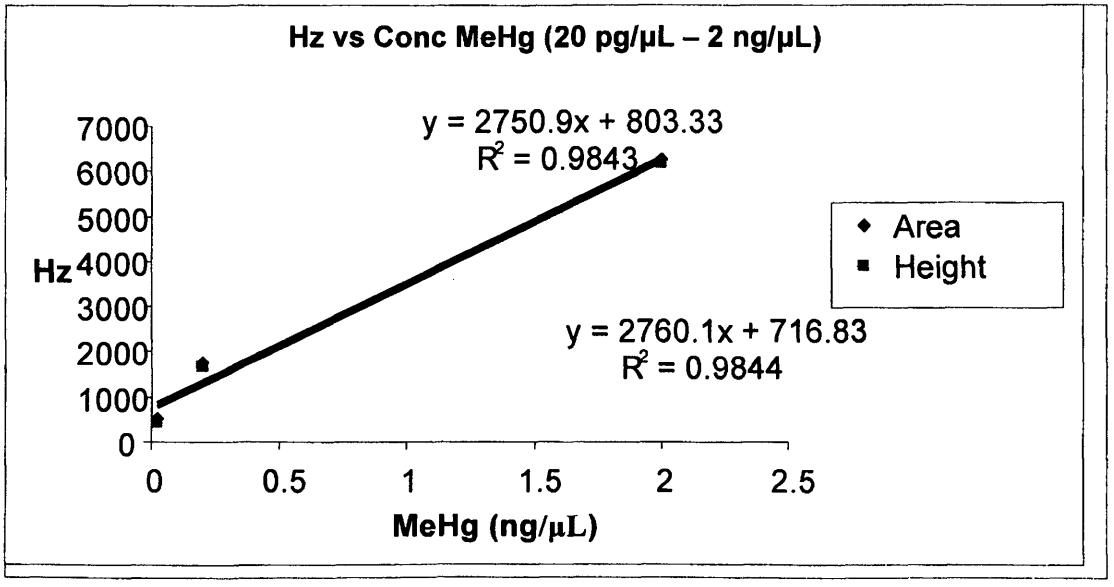


Figure 19. ECD extended calibration #2 for sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent. Graphs of both entire calibration and lower concentrations.

MeHg (ng/µL)	Area (Hz)	Height (Hz)	A ratio	H ratio
20	69359	61483	11.1	9.9
2	6269	6201	3.6	3.7
0.2	1751	1666	3.5	4.1
0.02	497	411		
0.002	2233	2103	← contamination	
0.0002	0	0		



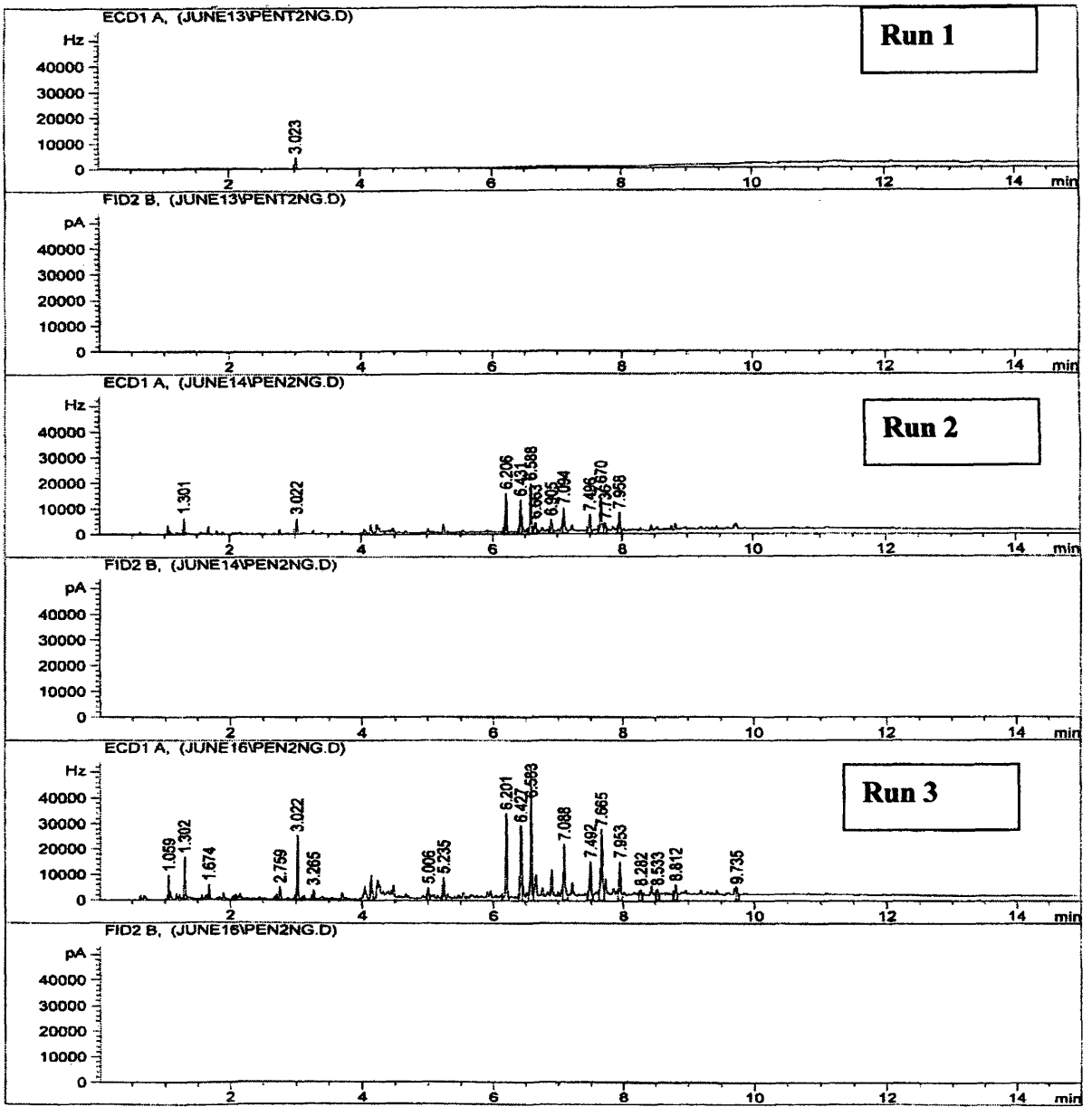


The first calibration used the reagent solution without mixing. In other words, the 200 μL of the 1% solution was removed without including the small amount of solid at the bottom of the flask. After derivatization, a relatively clear and significant MeHg peak resulted. In the days following, the solution was sonicated, mixing in the unknown residual solid, producing a more complex chromatogram with a smaller MeHg peak. A comparision showing the differences in the three runs is shown in Figure 20. Note that the peak at 3.02 minutes is assumed to be the pentafluorophenyl methylmercury derivative with a concentration of 2 ng MeHg per 1 μL injection. It is possible that sonicating the flask mixed in unwanted byproducts from the synthesis. This reagent, sodium tetrakis[pentafluorophenyl] borate, showed promise but it was evident that a cleaner synthesis was needed.

The first synthesis of the product was dissolved in methanol because TFPB had been more soluble in methanol than water. The Encyclopedia of Organic Compounds reported sodium tetrakis[pentafluorophenyl] borate as being completely soluble in water. The solid was then dissolved in water using a sonicator, but as with methanol, it did not completely dissolve. Once run on the GC-ECD, the chromatogram exhibited a large number of peaks, and a peak at 3.015-3.018 minutes possibly overshadowed the smaller mercury peak at 3.02 minutes. The peak at 3.02 minutes was only observed at an absolute concentration of 2 ng or higher.

A different temperature ramp (10°C/min instead of 20°C/min) was also investigated to determine if the peaks could be separated more. The new retention time at 10°C/min was 4.187 min, as this was the only peak that showed a difference between 20 ng/μL and 2 ng/μL. However, from 2 pg/μL -2 ng/μL, all peak areas were approximately

Figure 20. A comparison of three different runs of 2 ng absolute MeHg derivatized with sodium tetrakis[pentafluorophenyl] borate on GC-ECD. **1)** Run 1, derivatizing agent used without mixing – shows definite clean methylmercury peak at 3.023. **2)** Run 2, derivatizing agent used after sonication – shows many more peaks, most likely from byproducts but methylmercury peak still evident at 3.022. **3)** Run 3, derivatizing agent used with sonication, similar to Run 2.



the same. It was determined that the slower ramp time did not improve results. The final synthesis reported with 68% yield would prove more promising.

Derivatization optimitzation

pH

A study in *Chromatographia* reported that the pH of the buffer solution should be around 5 for TPB[16], but it was important to determine if this was also optimal for derivatization with TFPB. Buffers of pH 1, 2, 3, 5, 6.86 and 9 were obtained or prepared and used in conjunction with 20 ng MeHg /μL for derivatization. The change in peak area with varying pH is shown in Figure 21. The optimal pH was 5, while buffers of pH 1 and 9 apparently were incompatible for derivative formation given that no signal was observed.

Standard MeHgCl Degradation

It was important to determine the length of time a MeHgCl solution could be used and still give consistent peak areas. A four day study found that after the first day, a 20 ng/μL sample degraded by about 75% as shown in Figure 22. The third day showed slight increase from day two, possibly from contamination.

Figure 21. pH optimization using sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate as a derivatizing agent- peak area vs. pH.

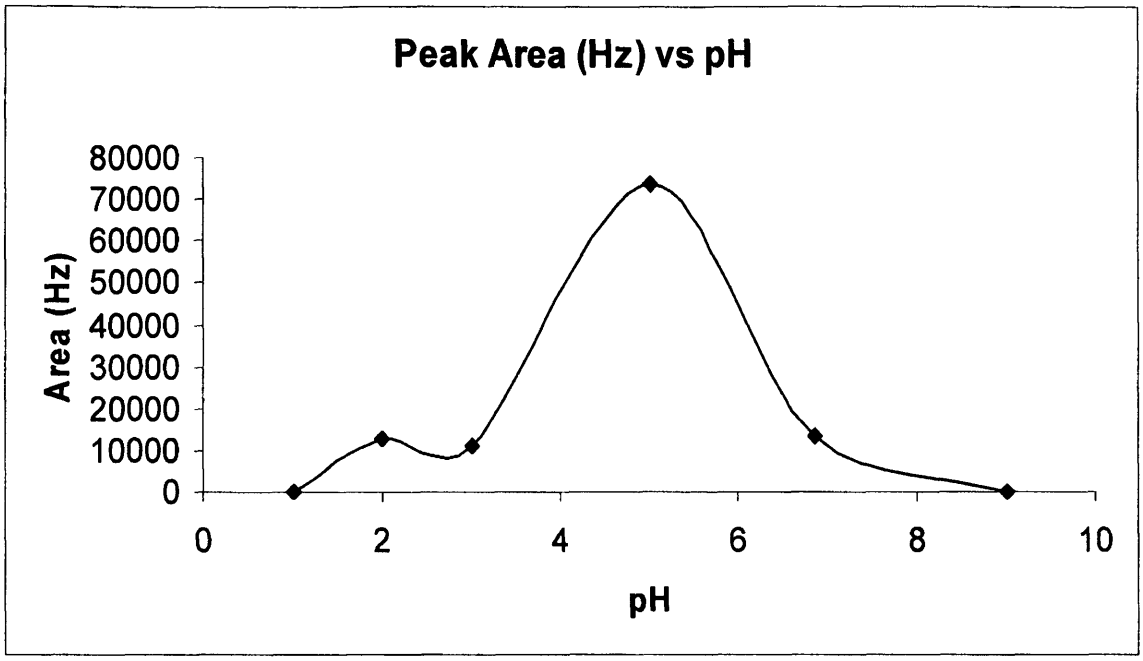
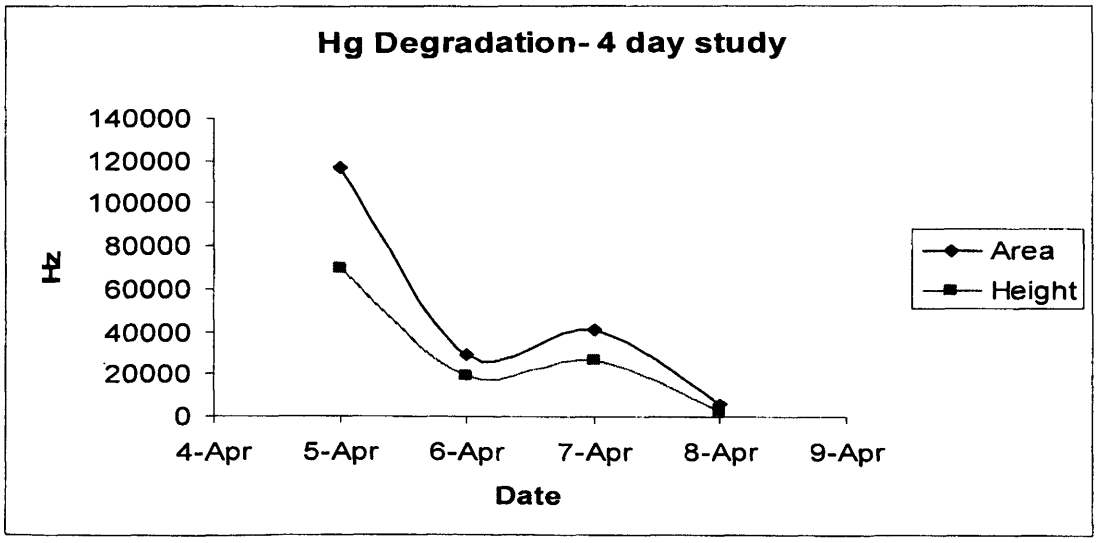


Figure 22. Hg degradation over four days using a 20 ng MeHg/μL sample



Solvents

Due to the insolubility of TFPB in water, it was possible that using water in the reaction hindered the derivatization. Deionized water was replaced with methanol so that the reaction vial contained 1 mL methanol, 1 mL buffer, 1 mL hexanes, 200 µL TFPB and 100 µL MeHgCl in methanol. Replacing deionized water with methanol was found to decrease peak areas and therefore did not improve results.

Hexanes had previously been used to extract the derivatized sample prior to running on the GC-ECD. Other solvents were examined to determine if there was possibly a solubility issue with dissolving all of the bis(trifluoromethyl)phenyl methylmercury in hexanes. The solvents initially compared were hexanes, ether and ethyl acetate. Hexanes were still found to be the optimal extraction solvent of the three.

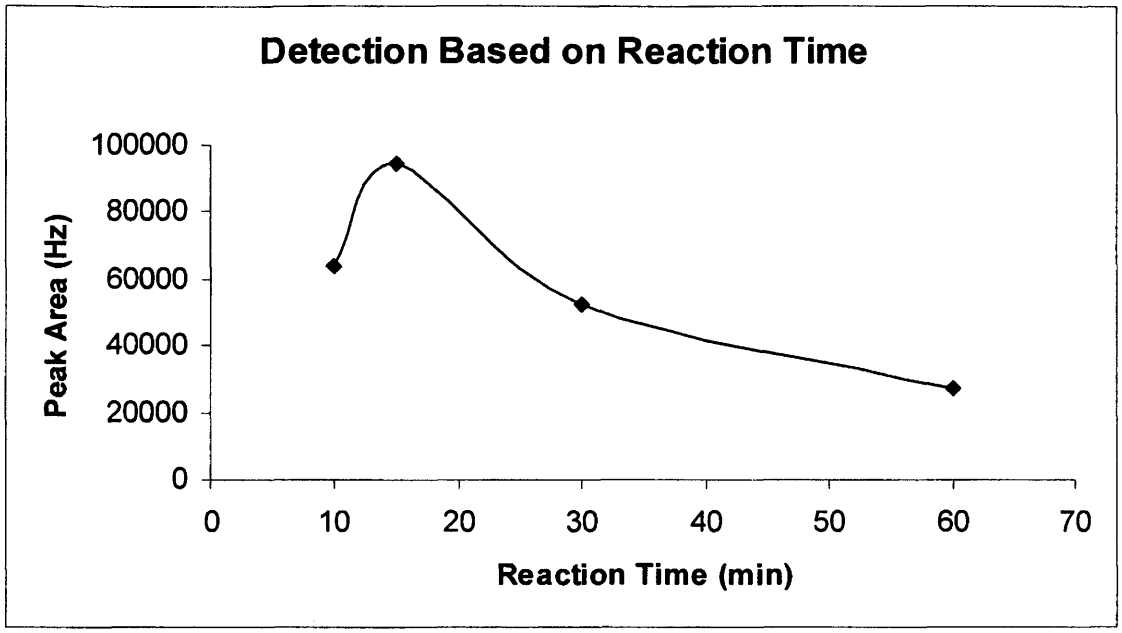
Benzene, isooctane, toluene and again, hexanes were then tested. Once the reaction vials were shaken, the aqueous layer of those containing benzene and toluene was milky and opaque. This however, clearly separated from the organic layer once centrifuged. All peaks eluted around 3.53 minutes as usual, although the benzene chromatogram had a large peak at 3.55 minutes that was determined to be from the solvent and not from the methylmercury derivative. This could overlap the mercury peak, therefore benzene was ruled out as a suitable solvent. The majority of the smaller peaks in the chromatogram were the same for all solvents, therefore these were a result of the derivatizing agent and not the solvent used. The peak areas at 3.53 minutes were approximately the same for the other three solvents, with isooctane was slightly better but not enough to make a significant difference.

Reaction Time

Derivatization reaction times of 10 minutes for TPB were recommended by Cai *et al.*[18] Various reaction times were run to determine the most optimal for TFPB. A sample shaker was set at 10, 15, 30 and 60 minutes for samples with 20 ng absolute MeHg for a 1 μL injection. A reaction time of 15 minutes was found to be optimal as shown in Figure 23.

A new calibration was performed after the reaction conditions were optimized; however unsatisfactory results were still obtained. Lower concentrations did not give expected peak areas and no peak was observed for the 2 pg/μL sample. The peak for 20 pg/μL was small but not in a correct ratio with the 200 pg/μL sample, so it was unclear as to whether or not the 20 pg/μL peak was reliable. It was then concluded from the data that either more optimization or another derivatization compound was needed with greater sensitivity.

Figure 23. Reaction time optimization using sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate as a derivatizing agent.



GC - Electron Capture Optimization

Detector Gas

Due to initial availability, the ECD make-up gas used filtered nitrogen, although it was reported that argon containing 5% methane was better for a pulsed detector versus a direct current detector with respect to sensitivity and linear results. Figure 24 shows the results of a calibration of TFPB prior to switching to Ar/CH_4. Results were unreliable with respect to peak areas, as area ratios should have been closer to 10. Figure 25 exhibits the same calibration after changing to Ar/Me, but prior to adding a filter as it was on order at the time.

Ratios were better for 2 ng/μL to 200 pg/μL, but were not as predicted for 200 pg/μL to 20 pg/μL, although 20 pg/μL did show up on the chromatogram this time. The addition of a filter was needed to give more accurate results.

As mentioned previously, a pure injection of trifluoroacetophenone in hexanes was run on the GC-ECD in order to observe the number of peaks produced. Trifluoroacetophenone was diluted in hexanes to make two samples, one 5 μg/mL and the other 500 ng/mL resulting in 5 ng and 500 pg absolute for a 1 μL injection. The samples were run using nitrogen and then Ar/Me in order to compare the two chromatograms. The peak representing the compound is most likely at 1.16 minutes, and is visible in both chromatograms when Ar/Me was used, but only the higher concentration when N_2 was used. There was also a higher number of peaks and more noise using nitrogen. It was determined that the chromatograms with large numbers of peaks from previous compounds were due to using nitrogen as a make-up gas, as well as the presence of byproducts.

Figure 24. ECD calibration of [3,5-bis(trifluoromethyl)phenyl] methylmercury as a derivatizing agent, with filtered nitrogen as a make-up gas.

Nitrogen (before Ar/Me)				
MeHg (ng/µL)	Area (Hz)	Height (Hz)	Ratio A	Ratio H
0.02	0	0		
0.2	87.6	141.6	17.1	9.5
2	1498	1338		

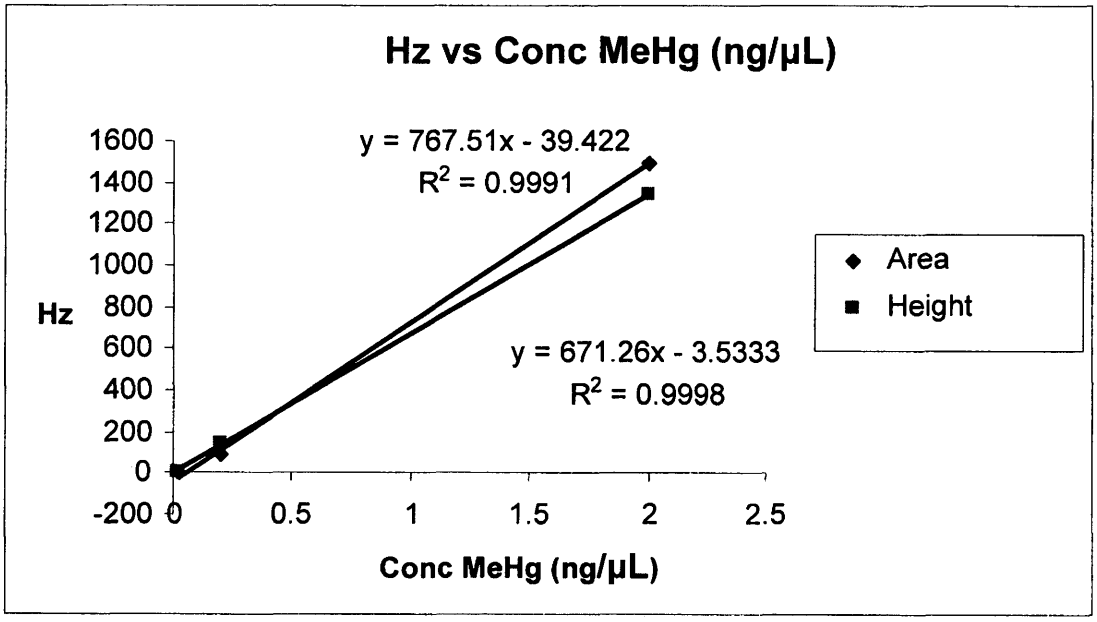
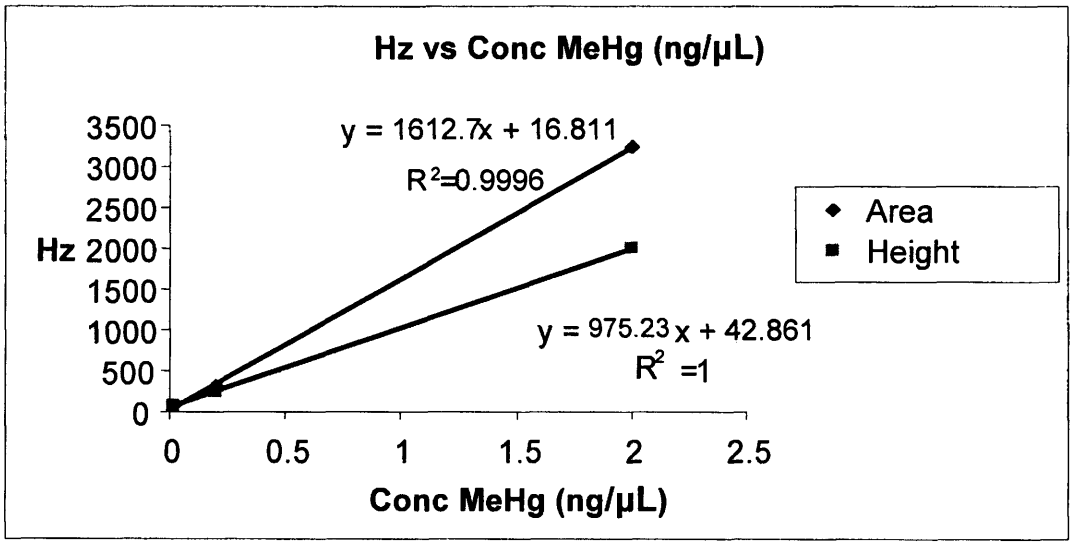


Figure 25. ECD calibration of [3,5-bis(trifluoromethyl)phenyl] methylmercury as a derivatizing agent, with unfiltered Ar / 5% Methane as a make-up gas.

Argon/Methane TFPB w/ MeHg

Conc MeHg (ng/µL)	Area (Hz)	Height (Hz)	Ratio A	Ratio H
0.02	83.2	64.1	3.6	3.7
0.2	301.8	236	10.8	8.4
2	3245.6	1993.5		



Sodium Tetrakis[pentafluorophenyl] borate using ECD with Ar/Methane

A calibration using unfiltered Ar/Methane as a make-up gas, and the product from the final synthesis of sodium tetrakis[pentafluorophenyl] borate gave the most promising results thus far as shown in Figure 26. There was still a relatively high blank, possibly from contamination, and detection of the derivative started at approximately 20 pg/μL. Further testing and optimizations followed.

When previous derivatizing reactions were carried out, separate solutions of MeHgCl in methanol were produced for each concentration needed and all derivatizations were carried out separately. It was possible that results were unsatisfactory due to experimental errors in dilutions of MeHgCl. A new calibration was carried out, shown in Figure 27, with dilutions made from the already derivatized 20 ng MeHg/μL sample. These results showed little improvement, leading to the conclusion that it did not matter when the dilutions were made- prior to or after the reaction of MeHgCl and the derivatizing agent. Shortly after this calibration was run, the GC column was clipped, shortening the retention time from 2.55 to 2.50 minutes.

Figure 26. ECD calibration #1 of pentafluorophenyl methylmercury, final synthesis with unfiltered Ar/5% Me as a make-up gas.

Peak (min)	Conc MeHg (ng/µL)	Peak Area (Hz)	Ratio
2.552	0	890.7	1.1
2.552	0.002	954.6	1.1
2.552	0.02	1057.1	2.0
2.553	0.2	2166.9	17.8
2.553	2	38523.6	7.1
2.553	20	273718	

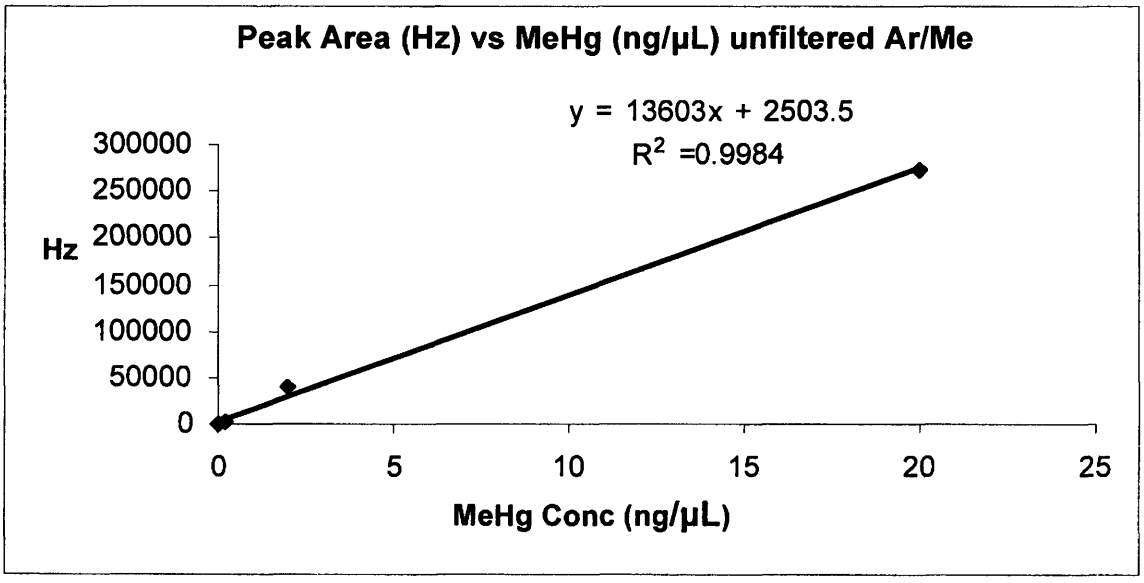
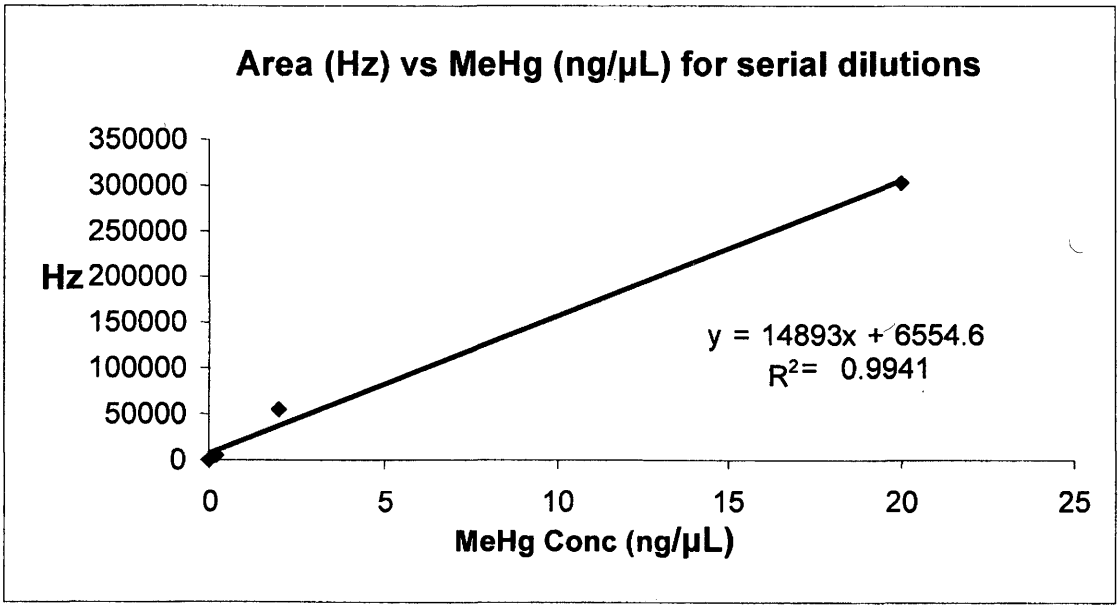


Figure 27. Calibration #2 from final synthesis of pentafluorophenyl methylmercury, with serial dilutions of derivatized product, using unfiltered Ar/5% Me as make-up gas.

Peak (min)	MeHg Conc (ng/µL)	Peak Area (Hz)	Ratio
2.552	0.002	346.326	2.4
2.553	0.02	836.768	7.0
2.553	0.2	5846.23	9.2
2.552	2	54004.5	5.6
2.554	20	302696	



Optimization of Additional GC-ECD Parameters

The carrier gas (He) of the GC is important in peak resolution and peak width. The resolution is how well the peaks are separated at the baseline, while the width of a peak determines how efficient the column is. It is important to optimize column flow in order to produce peaks in a timely manner, while still maintaining peak resolution. The make-up gas (detector gas) flow rate affects peak shape, linearity and reproducibility. It is inversely related to ECD sensitivity, but it is also important to have the flow high enough to sweep highly retained substances through the detector in order to produce definite peaks.[26] These parameters are important for attaining sharp peaks with adequate resolution and sensitivity. A 2 ng MeHg absolute injection using sodium tetrakis [pentafluorophenyl] borate as the derivatizing agent was prepared and tested at various make-up flows, and then with varying column flows to determine the optimal settings. Make-up flow is shown in Figure 28 while column flow is shown in Figure 29. The original setting was a flow of 40 mL/min combined for both the make-up flow and column flow, with the column flow set at 10.5 mL/min. It was determined that a make-up flow of 30 mL/min and a column flow of 16 mL/min were optimal.

The Agilent software recommended that the make-up flow to the detector not go below 10 mL/min, and stated that the norm is 60-30 mL/min. After testing various make-up flows from 60 to 30 mL/min, the flow was dropped further while holding the column flow constant to see if this could increase detection even more. Lower makeup flow results are shown in Figure 30. The flow was dropped down to 8 mL/min at the minimum, just to observe if there was a difference between 10 mL/min and 8 mL/min.

Figure 28. Make-up flow setting vs. peak area for 2 ng MeHg/μL sample using sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent. Column flow set at 10.5 mL/min.

Make-up flow	Area (Hz)
60	11583.2
40	12185.9
30	20988.8

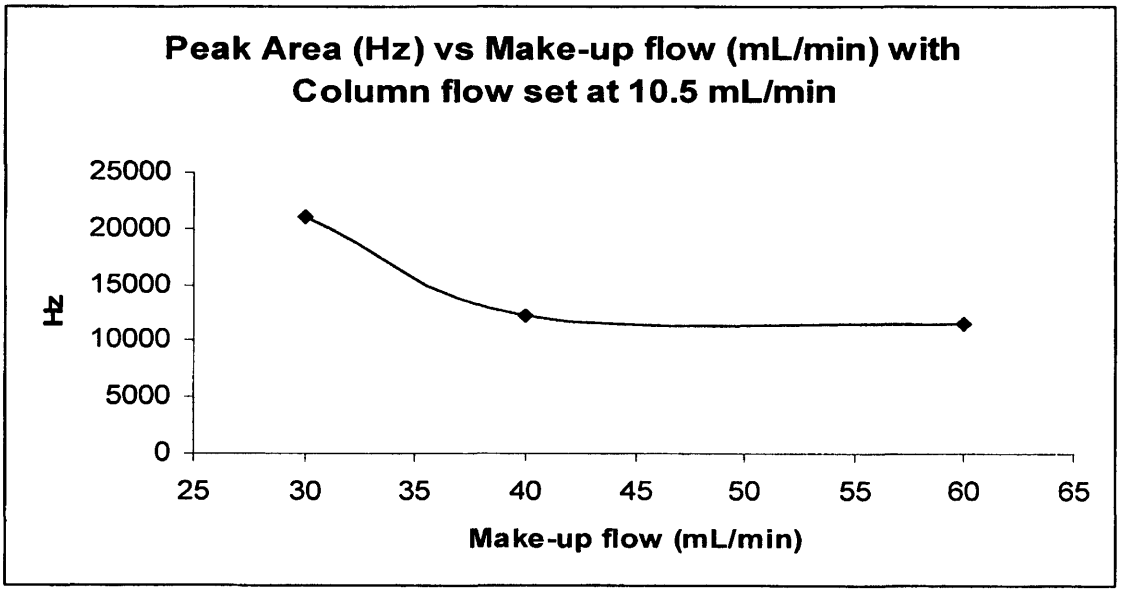


Figure 29. Column flow setting vs. peak area for 2 ng MeHg/μL sample using sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent. Make-up flow set at 30 mL/min.

Column Flow	Peak	Peak Area
14	2.281	20204
15	2.233	27117.8
16	2.188	28961.4

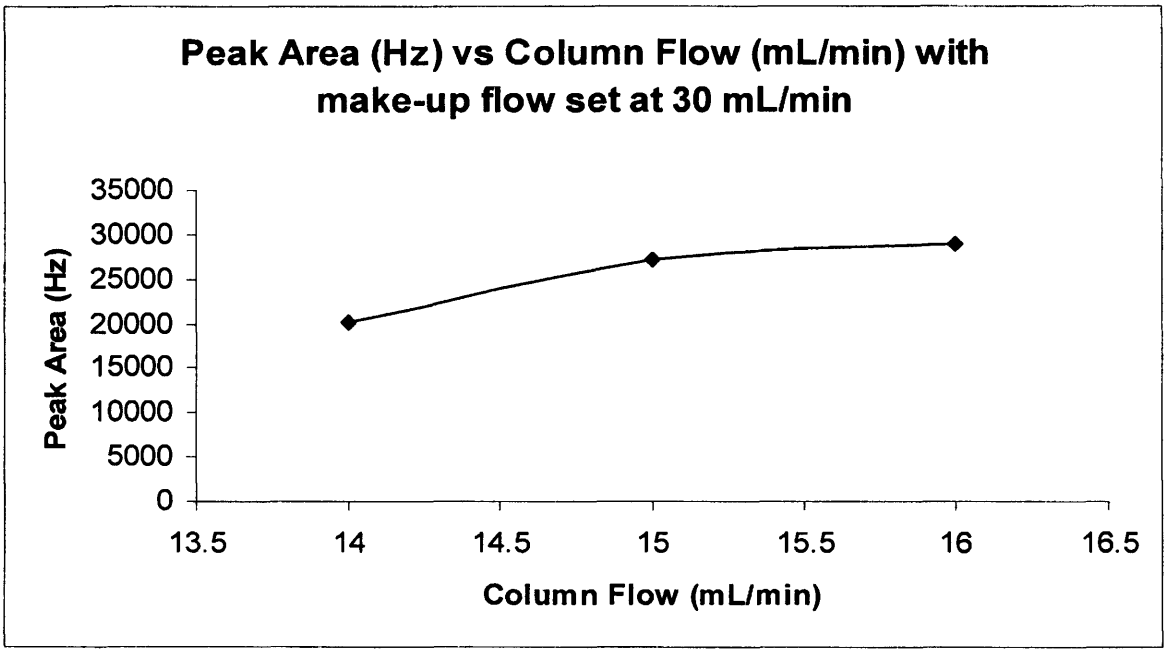
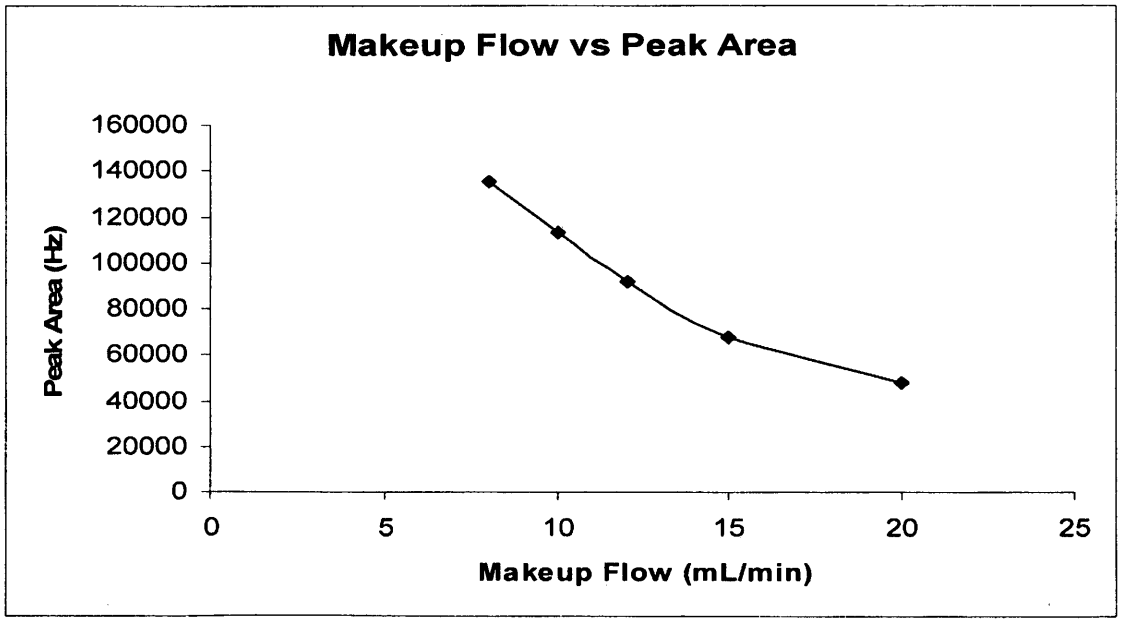


Figure 30. Make-up settings (mL/min) vs. peak area from 20 to 8 mL/min with a 2 ng/μL MeHg sample using sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent. Column flow set at 16 mL/min.

Makeup Flow (mL/min)	Peak Area (Hz)
20	47770.8
15	67847.1
12	91520.5
10	113293
8	135170

(recommends to only go down to 10 at lowest)

keep at 10mL/min makeup flow with 16 mL/min column flow



It was determined that the optimal makeup and column flow settings were 10 mL/min and 16 mL/min respectively. Once flows were optimized, a calibration of 2 pg/μL – 20 ng/μL was performed and produced promising results, shown in Figure 31 and Figure 32, the latter not including 20 ng/μL to observe the linearity at lower concentrations.

The next parameters to test were the inlet and detector temperatures. The inlet temperature had previously been set to 200°C because this was the default setting on the GC-MS. Other temperatures were tested as shown in Figure 33, with 190°C found to be the most favorable. It had been previously set at 300°C. Detector temperatures were tested from 300°C to 320°C and peak areas were tabulated. The optimal temperature appeared to be 310°C.

The final parameters for optimizing the derivatized signals were as follows:

190°C inlet temperature
10 mL/min makeup flow
16 mL/min column flow
310°C detector temperature

A final calibration using the optimized parameters was performed, with the results shown in Figure 34 and Figure 35 showing the lower end of the calibration.

IV. Applications

In order to check the validity of the changed parameters and the final calibration reported in Figure 34, a more extensive calibration was made within the same concentration range but with more standards. The results are presented in Figure 36 and 37. Both the entire calibration and the lower end of the calibration had R^2 values of

Figure 31. Peak area (Hz) vs MeHg (ng/μL) after optimization of flows using sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent.

Conc (ng)	Peak Area	Ratio
0	0	
0.002	206.3	7.3
0.02	1508.68	9.4
0.2	14138.9	7.8
2	109868	8.1
20	886358	

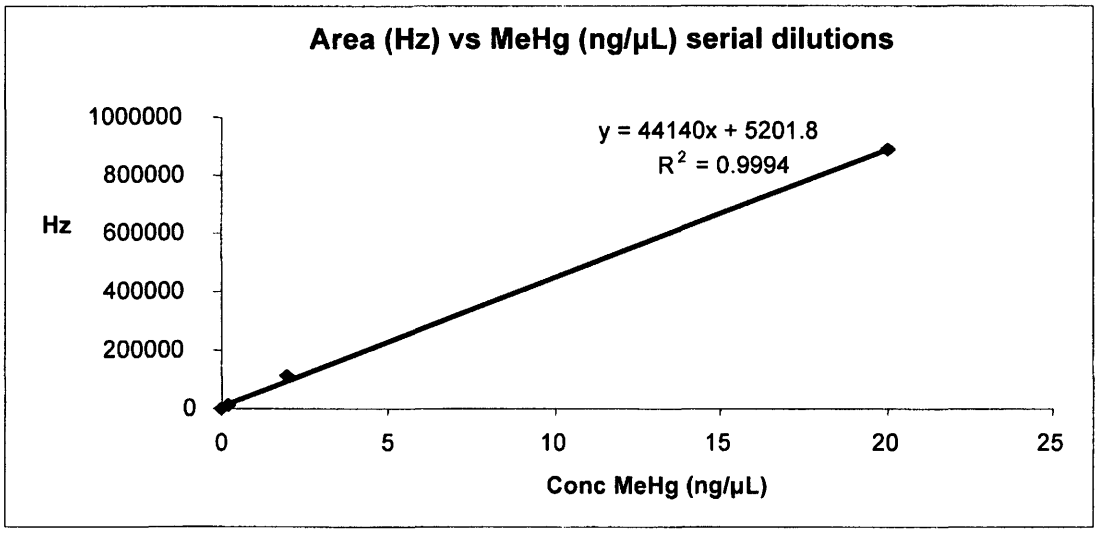


Figure 32. Peak area (Hz) vs MeHg (ng/μL) after optimization of flows using sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent. From 2 pg MeHg/μL - 2 ng MeHg/μL to show linearity at lower concentrations.

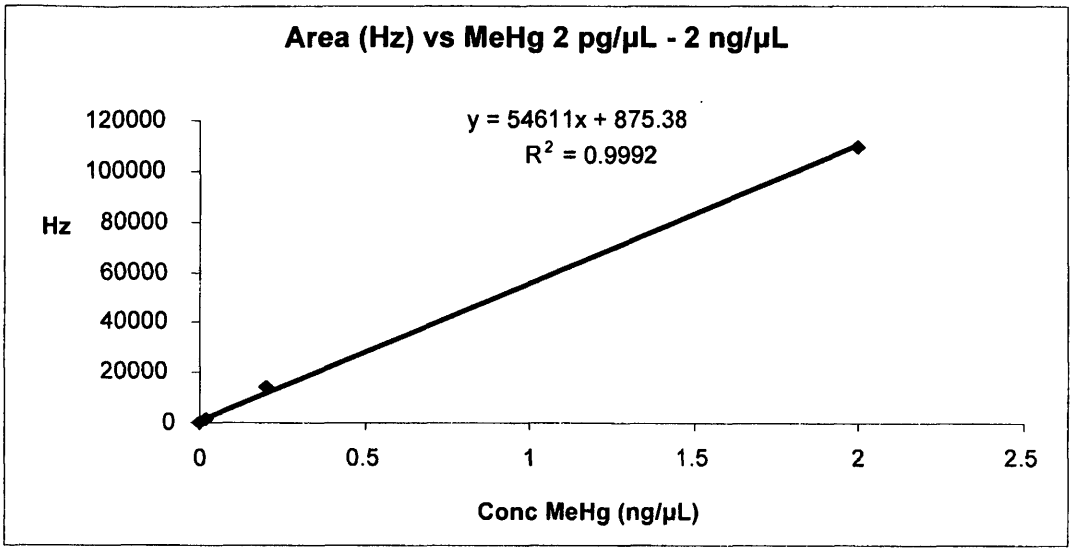


Figure 33. Peak area (Hz) as a function of injection port temperature from 180°C to 220°C using a 2 ng MeHg/μL sample derivatized with sodium tetrakis [pentafluorophenyl] borate.

Injection Temp (°C)	Peak Area (Hz)
180	98537.7
190	139772
200	84390.3
210	83308.4
220	93489.4

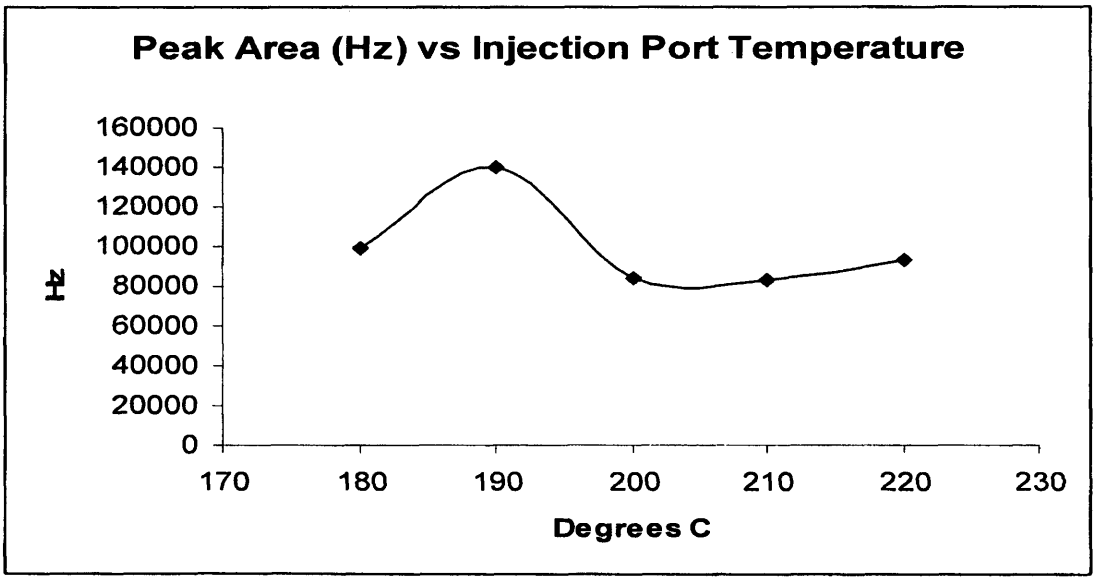


Figure 34. Calibration of pentafluorophenyl methylmercury from 2 pg MeHg/μL -20 ng MeHg/μL after optimization of parameters.

Peak (min)	Hg Conc (ng/μL)	Peak Area (Hz)	Ratio
2.185	0.002	189.361	8.1
2.187	0.02	1530.92	10.8
2.188	0.2	16500.2	5.9
2.188	2	96775.4	5.7
2.19	20	550306	

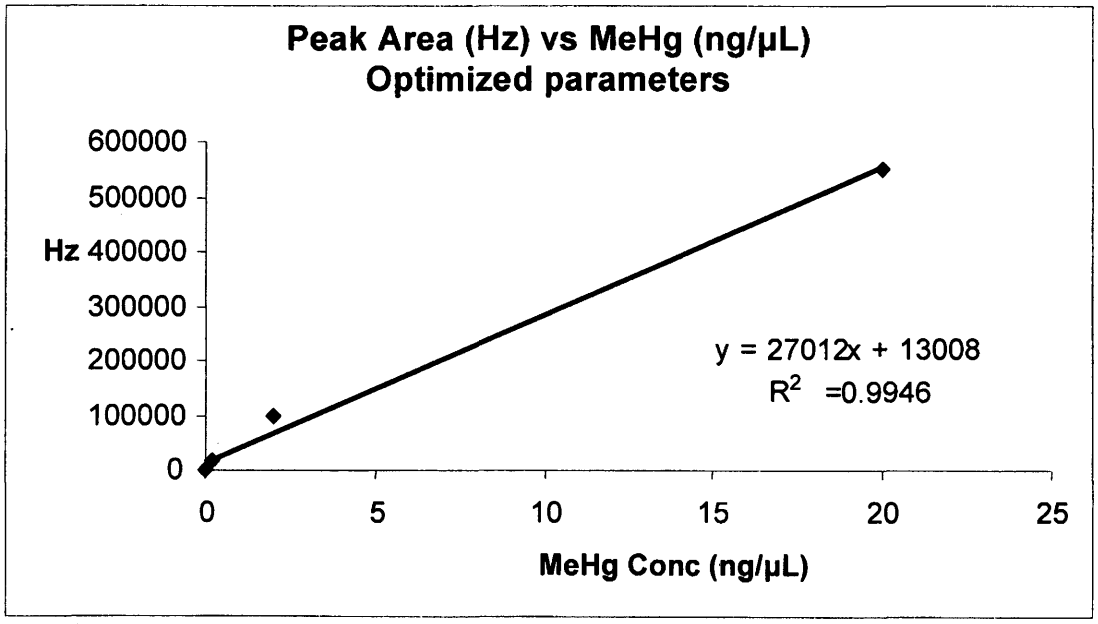


Figure 35. Calibration of pentafluorophenyl methylmercury from 2 pg MeHg/μL - 2 ng MeHg/μL after optimization of parameters.

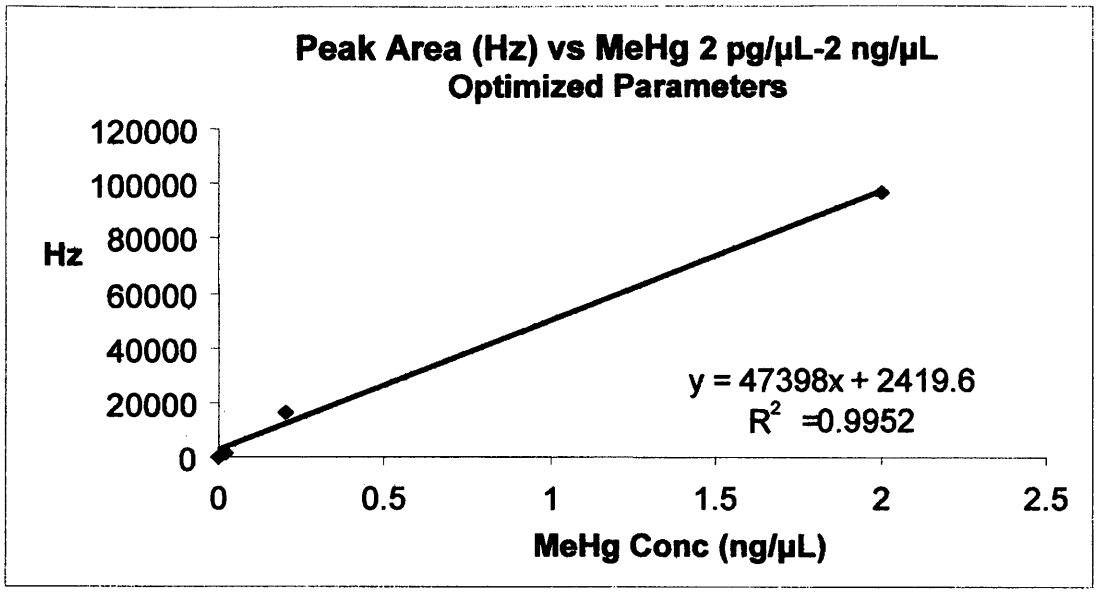


Figure 36. Extended calibration of pentafluorophenyl methylmercury from from 2 pg MeHg /μL -20 ng MeHg/μL after optimization of parameters.

Conc MeHg (ng/μL)	peak area (Hz)	ratio	correct ratio
0.002	404.523	2.1	2.50
0.005	838.345	2.5	4.00
0.02	2062.09	2.7	2.50
0.05	5470.72	3.1	4.00
0.2	17167.7	2.9	2.50
0.5	49414.6	2.8	4.00
2	136063	2.1	2.50
5	283395	1.5	2.00
10	427405	1.9	2.00
20	815511		

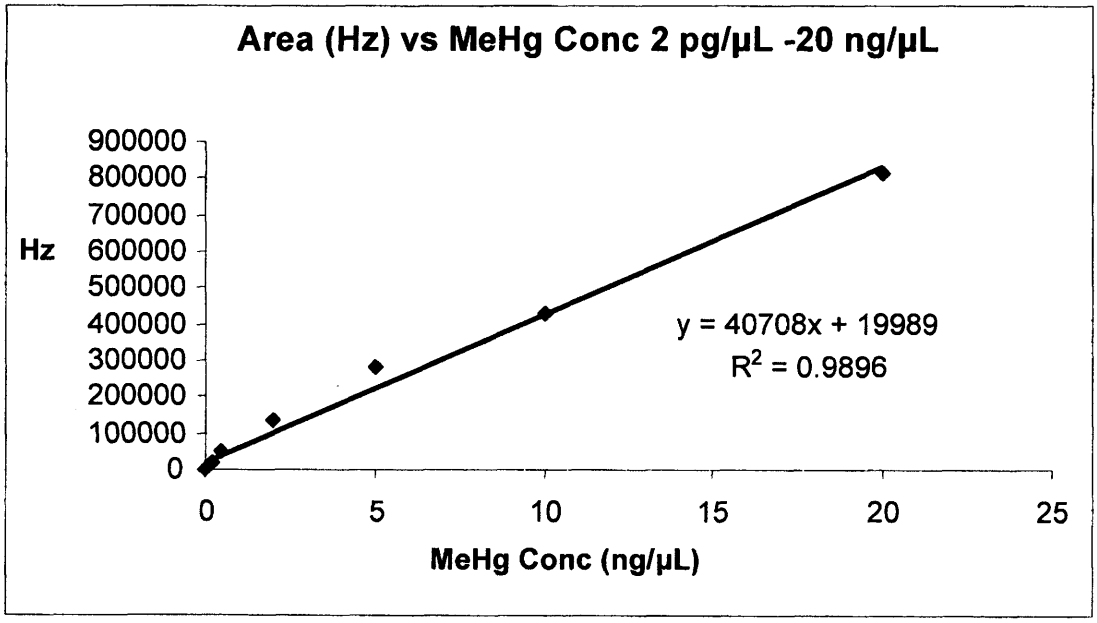
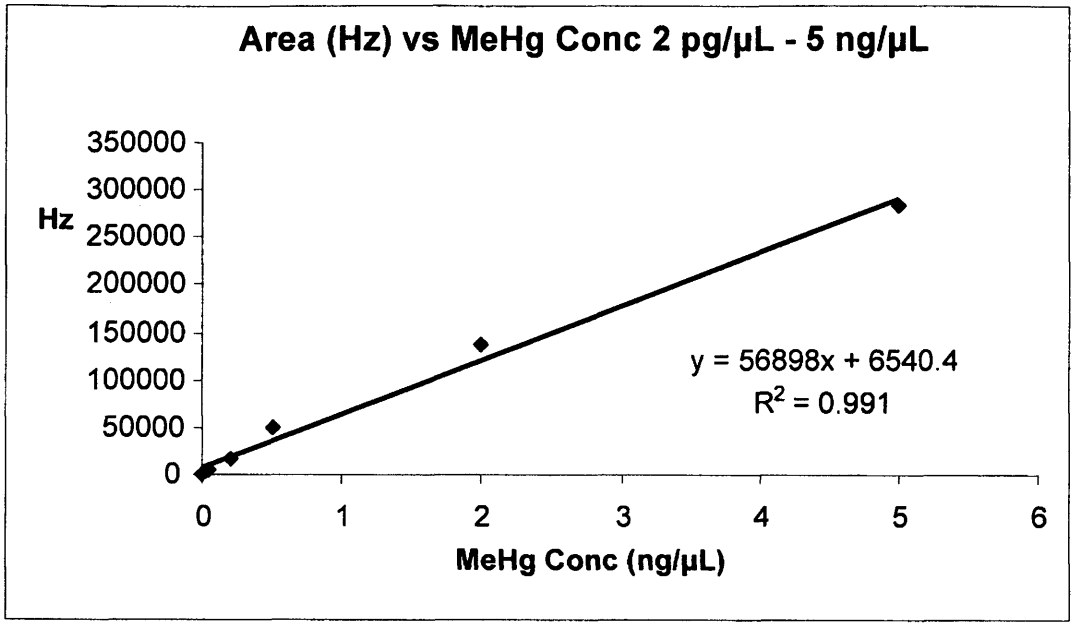


Figure 37. Extended calibration of pentafluorophenyl methylmercury after optimization of parameters, examining lower end of calibration from 2 ppb - 5 ppm MeHg.



The limit of detection for the use of sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent for the detection of methylmercury was found to be ~0.8 pg MeHg/μL. The correlation coefficient of a calibration from 2 pg to 20 ng per 1 μL injection (2 ppb – 20 ppm), was found to be 0.9896, and when examining the same calibration at the lower end of the spectrum, from 2 pg to 5 ng per 1 μL injection (2 ppb – 5 ppm), the linearity was somewhat better giving a correlation coefficient of 0.991.

Further testing would be required to fully investigate the potential of sodium tetrakis[pentafluorophenyl] borate as a derivatizing agent. A considerable amount of time was devoted to developing the synthetic strategies and techniques for the derivatives as well as optimization of the parameters of the GC-ECD instrument recently purchased by the department. Recent developments in fish digestion techniques allowed for one application of the derivatizing agent to fish tissue methylmercury determinations.

Certified Fish Tissue Digestion and Derivatization

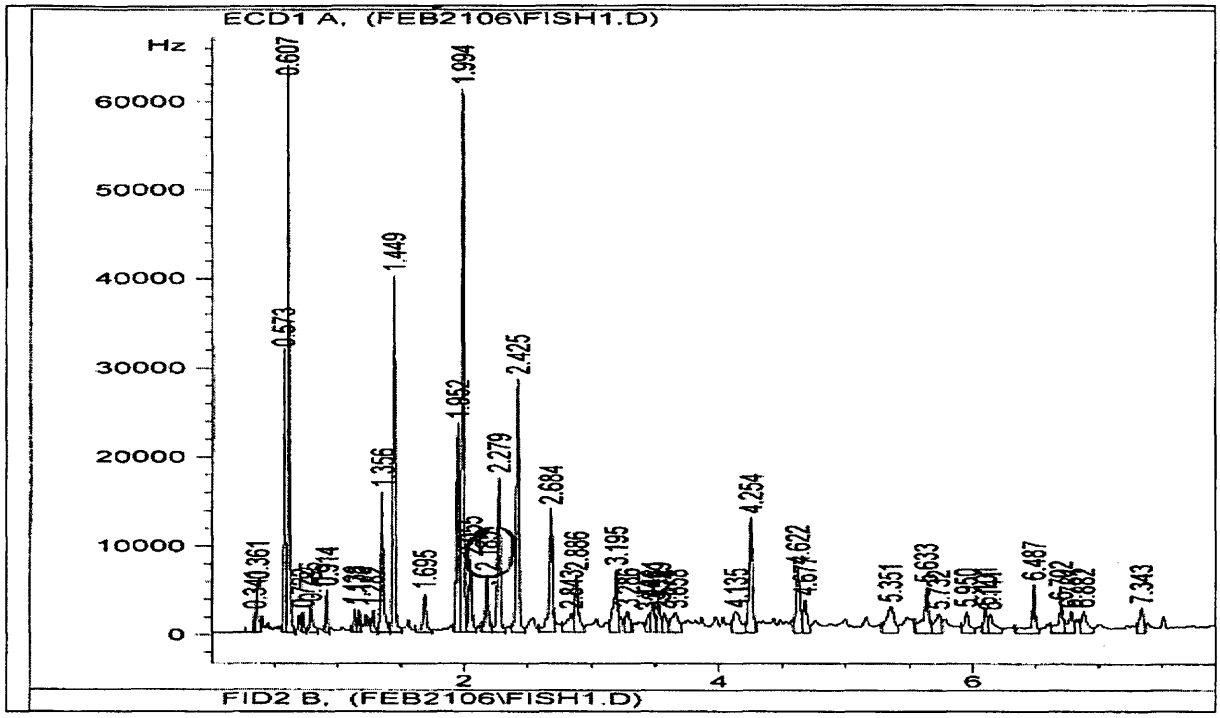
A digestion for fish tissue developed by Justin Williams as part of his senior honors thesis work was used to determine if sodium tetrakis[pentafluorophenyl] borate would be a suitable derivatizing reagent for the determination of methylmercury in fish tissue. A certified dried dogfish muscle sample (DORM-2, National Research Council of Canada) was purchased in order to determine the accuracy of methylmercury recoveries.

A 0.05 g certified sample of the dried tissue was weighed directly into a Teflon microwave vessel prior to the addition of 10.0 mL of 6 M NaOH. The tube was closed tightly and the fish digested using a microwave digestion system (MARS Express, CEM Corp.). The microwave temperature was ramped to 100 °C at 1200 W over 2.5 minutes,

A 0.05 g certified sample of the dried tissue was weighed directly into a Teflon microwave vessel prior to the addition of 10.0 mL of 6 M NaOH. The tube was closed tightly and the fish digested using a microwave digestion system (MARS Express, CEM Corp.). The microwave temperature was ramped to 100 °C at 1200 W over 2.5 minutes, held at 100 °C for an additional 8.5 minutes, followed by cooling for an additional 5 minutes. Once the vessels cooled to room temperature, a 5 mL aliquot of the digested solution was transferred into a 15 mL plastic vial along with 1 mL of 0.10 M manganese acetate, 1 mL of concentrated acetic acid to adjust the pH to 4-5, 1 mL of 1% derivatizing solution, and 1 mL of hexanes. The vial was shaken on a mechanical shaker for 35 minutes and centrifuged for 2 minutes to ensure adequate separation of the organic and aqueous layers. The hexane layer was transferred using a Pastuer pipet into a 2 mL amber vial and sodium sulfate added to remove residual water.

The chromatogram obtained using the optimized GC-ECD conditions previously described is shown in Figure 38. The eluting time for the derivatized methylmercury in previous standards was 2.19 minutes. There is a definite peak at 2.18 minutes thought to be the desired derivative; however there was some uncertainty as to whether or not this was the expected peak since it had been some time since the system had been operated for the specific determination of this derivative. There were a significant number of large peaks in the chromatogram, assumed to be the result of other electrophilic groups extracted from the dogfish tissue. Nevertheless, the peak assumed to be the derivative was clearly resolved from the other non-analyte constituents extracted from the fish tissue.

Figure 38. ECD chromatogram obtained from derivatization of methylmercury in DORM-2 fish tissue using sodium [pentafluorophenyl] borate. The peak at 2.185 represents the methylmercury derivative.

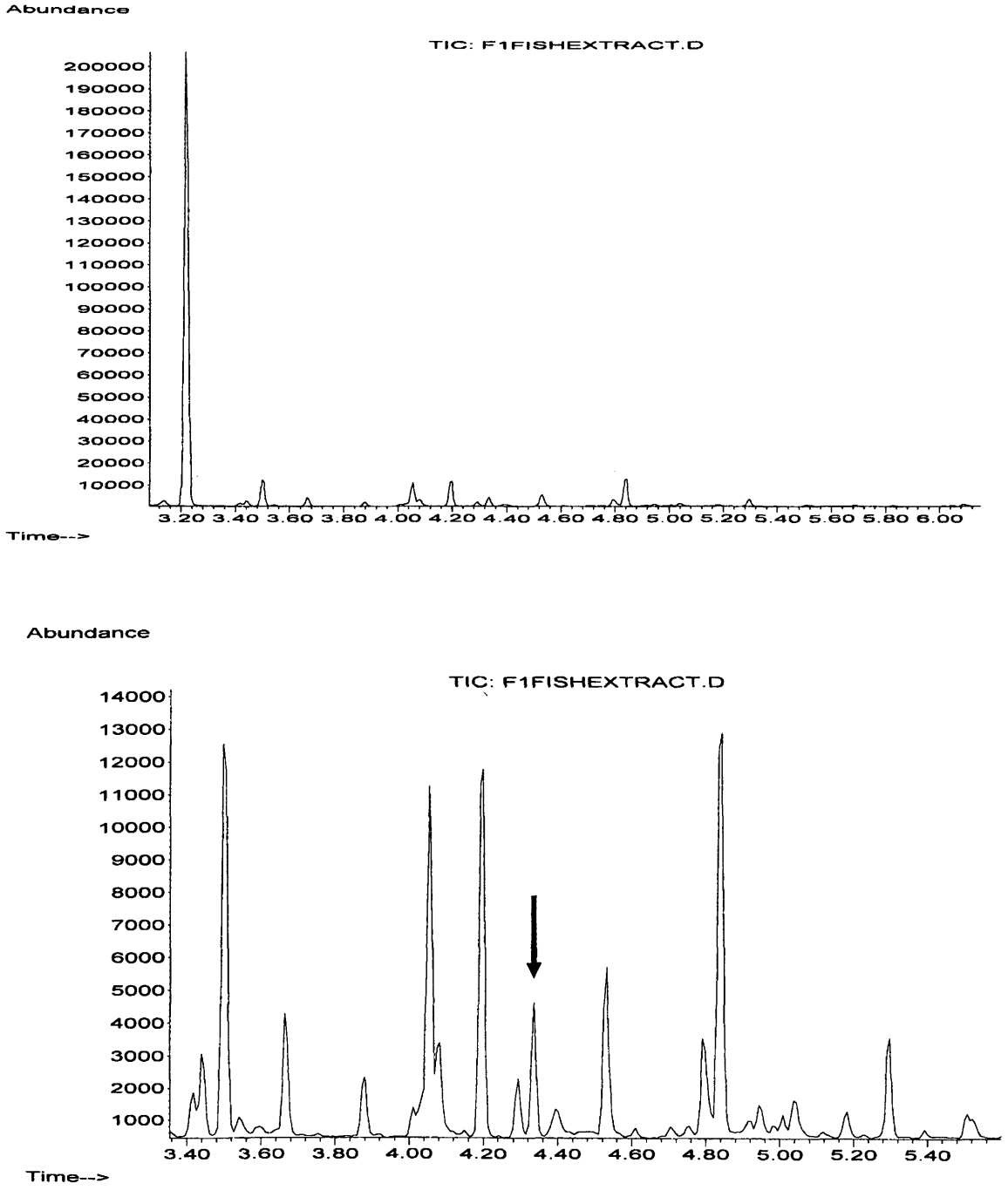


The same derivatized dogfish sample was then injected on the GC-MS in an ion selective mode (117 and 167 amu) to confirm the ECD observations. The ion selective mass spectrum is shown in Figure 39. The peak at 4.35 minutes indeed confirmed that the reagent did produce the methylmercury derivative from the fish tissue. The concentration was estimated to be approximately 0.10 ug/mL based on recently obtained

calibrations using standard derivatizations of methylmercury chloride standards, which correlated to 4 ug MeHg /g fish tissue. According to the certification documentation (4.7 ug/g), this resulted in ~85% recovery.

It was surprising that there were a number of significant peaks observed in the GC-MS chromatogram in the ion selective mode comparable to the ECD chromatogram. This implies that there must be a significant number of species within the extracted fish tissue that are potentially producing derivatives with the derivatizing reagent given that the 167 amu ion, representing the pentafluorophenyl fragment, would not be expected to be a prominent ion from other species. Given that a significant number of digested species could potentially be extracted as well, there is still the remote possibility that the ion fragment is being generated in another form. The other observation that can be noted in the top chromatogram is the very large peak at ~3.2 minutes, which had previously been identified as the decafluorobiphenyl by-product. Although this peak is observed in relatively small amounts from derivatization of standard methylmercury solutions, the formation of this by-product is far more pronounced from the fish derivatives, again implying that there are more species in the extracted solution reacting with the derivatizing agent than the analyte species.

Figure 39. Chromatogram obtained from the GC-MS in an ion selective mode for the derivatized fish extract. Top chromatogram from 3-6 minutes shows the large decafluorobiphenyl by-product at 3.2 minutes. Bottom chromatogram with enlarged analyte region (arrow denotes derivatized methylmercury peak).



Time restrictions only allowed for one testing of the application of the sodium tetrakis[pentafluorophenyl] borate as a derivatizing reagent with the certified fish tissue, but the early evidence indicated that it shows great promise for the determination of methylmercury in complex sample matrices. Further and more definitive work remains to be done in order to fully confirm the ability of GC-ECD to detect the accurately quantitate methylmercury in fish as well as possible extension into other types of biological or other matrices.

CONCLUSIONS and FURTHER RESEARCH

This study has examined the synthesis and potential merits of five polyfluorinated reagents as derivatizing agents for the detection of methylmercury using GC-ECD. The most successful compound overall was sodium tetrakis[pentafluorophenyl] borate, as it resulted in a relatively clean synthesis with high yields (68%), as well as providing an appropriate reagent for methylmercury detection through electron capture. The final cost of materials for the synthesis was somewhat higher than expected. Therefore, as it currently stands, the cost of the derivatizing agent will be higher than the more widely-used reagent, tetraphenylborate (TPB).

The GC-ECD, a sensitive, common, and low-cost detector shows promise as a means for methylmercury detection using polyfluorinated derivatives. Detection limits were in the range of low parts per million to low parts per billion. Methylmercury samples of 2 ppb to 5 ppm MeHg were detected using GC-ECD in conjunction with the synthesized derivatizing agents. Linearity was best in the range of low pg to low ng; delineating at higher concentrations around 20 ppm MeHg or higher.

Future research can be pursued in several directions. Further syntheses could be run in order to increase the purity of the polyfluorinated compounds, allowing for further ease of detection using GC-ECD by reducing the number of extraneous peaks observed in the chromatograms.

It is also possible that the synthesized polyfluorinated reagents could show promise in methylmercury detection through the use of GC-MS in the selective ion monitoring (SIM) mode. GC-MS was used in this study to characterize products, although varying concentrations and selectivity was examined in a limited manner. In the

past this method has shown promise as a means for methylmercury detection using tetraphenylborate, so it is assumed that it could be successful using sodium tetrakis [pentafluorophenyl] borate as well. The higher mass associated with the parent pentafluorophenyl ion could provide greater sensitivity and better selectivity relative to the phenyl ion used for TPB.

Due to time constraints, extensive testing of this procedure using fish tissue samples was not carried out beyond the initial tests reported. This is necessary in order to fully understand the possibilities of sodium tetrakis [pentafluorophenyl] borate as a derivatizing agent and GC-ECD as a means of detection. Current research in our lab is making ground on maximizing recoveries of methylmercury in fish tissue digestions using the more conventional phenyl derivative. Preliminary methylmercury detection performed on digested certified fish samples has shown promise using GC-MS and GC-ECD as earlier reported. While initial testing with this new derivatizing agent has shown great potential, further testing is needed to fully understand detection limits and linear ranges when applied to fish tissue.

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VITA

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Crystal went on to receive her Bachelor of Science in May of 2004 at the College of William and Mary in Virginia with a degree in chemistry. The following fall she started the graduate program in the chemistry department at William and Mary, under Dr. Gary Rice, and defended her thesis in February of 2006. She currently resides in Norfolk, VA.